=> fil hcaplu
FILE 'HCAPLUS' ENTERED AT 15:58:09 ON 09 AUG 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.

FILE COVERS 1907 - 9 Aug 2002 VOL 137 ISS 7
FILE LAST UPDATED: 8 Aug 2002 (20020808/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

CAS roles have been modified effective December 16, 2001. Please check your SDI profiles to see if they need to be revised. For information on CAS roles, enter HELP ROLES at an arrow prompt or use the CAS Roles thesaurus (/RL field) in this file.

=> d stat que
L1 1 SEA FILE=REGISTRY "MSP1 (PROTEIN) (PLASMODIUM VIVAX STRAIN V200 GENE MSP1 C-TERMINAL FRAGMENT)"/CN
L2 1 SEA FILE=REGISTRY IDE8/BI
L3 20 SEA FILE=HCAPLUS L1 OR MSP1A OR MSP1(W)A
L4 7 SEA FILE=HCAPLUS L2 OR IDE8 OR IDE(W)8
L7 2 SEA FILE=HCAPLUS (L3 AND L4) AND (VACCIN? OR ?IMMUN?)

=> d ibib abs hitrn l7 1-2

L7 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:353311 HCAPLUS
DOCUMENT NUMBER: 136:368445
TITLE: Recombinant major surface protein from Anaplasma marginale for **vaccination**
INVENTOR(S): De La Fuente, Jose De Jesus; Kocan, Katherine M.; Garcia-Garcia, Jose Carlos; Blouin, Edmour F.
PATENT ASSIGNEE(S): The Board of Regents for Oklahoma State University, USA
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002036159	A2	20020510	WO 2001-US48505	20011030

W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2000-244333P P 20001030

AB The authors disclose a **vaccine** prepn. for eliciting an enhanced **immune** response against Anaplasma marginale. The **vaccine** comprises recombinant **MSP1a** surface protein alone or in combination with tick cell culture-derived A. marginale.

L7 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:25019 HCAPLUS

DOCUMENT NUMBER: 130:194064

TITLE: Comparison of surface proteins of Anaplasma marginale grown in tick cell culture, tick salivary glands, and cattle

AUTHOR(S): Barbet, A. F.; Blentlinger, R.; Yi, Jooyoung; Lundgren, A. M.; Blouin, E. F.; Kocan, K. M.

CORPORATE SOURCE: Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32611-0880, USA

SOURCE: Infection and Immunity (1999), 67(1), 102-107
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Anaplasma marginale, a tick-borne rickettsial pathogen of cattle, infects bovine erythrocytes, resulting in mild to severe hemolytic disease that causes economic losses in domestic livestock worldwide. Recently, the Virginia isolate of A. marginale was propagated in a continuous tick cell line, **IDE8**, derived from embryonic Ixodes scapularis. Development of A. marginale in cell culture was morphol. similar to that described previously in ticks. In order to evaluate the potential of the cell culture-derived organisms for use in future research or as an antigen for serol. tests and **vaccines**, the extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. marginale and the bovine erythrocytic stage, currently the source of A. marginale antigen, was detd. Structural conservation on the tick salivary-gland stage was also examd. Monoclonal and monospecific antisera against MSPs 1 through 5, initially characterized against erythrocyte stages, also reacted with A. marginale from cell culture and tick salivary glands. **MSP1a** among geog. A. marginale isolates is variable in size because of different nos. of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. marginale

maintained the same-size **MSP1a** as that found on the Virginia isolate of A. marginale in bovine erythrocytes and tick salivary glands. Although differences were obsd. in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by two-dimensional gel electrophoresis, during continuous passage in culture. These data show that MSPs of erythrocyte-stage A. marginale are present on culture stages and may be structurally conserved during continuous culture. The presence of all current candidate diagnostic and **vaccine** antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and **vaccines** against anaplasmosis.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

```
=> d his

     (FILE 'HOME' ENTERED AT 15:47:31 ON 09 AUG 2002)

     FILE 'REGISTRY' ENTERED AT 15:47:43 ON 09 AUG 2002
                  E MSP1A/CN
L1              1 S E2
                  E IDE8/CN
                  E IDE 8/CN
                  E IDE 8
L2              1 S E5
                  E ANAPLASMA MARGINALE/CN

     FILE 'HCAPLUS' ENTERED AT 15:51:40 ON 09 AUG 2002
L3             20 S L1 OR MSP1A OR MSP1(W)A
L4              7 S L2 OR IDE8 OR IDE(W)8
L5            289 S ANAPLASMA OR MARGINALE OR L3
L6            176 S RUMINANTS/CV
                  E RUMINANTS+ALL/CV
L7              2 S (L3 AND L4) AND (VACCIN? OR ?IMMUN?)

     FILE 'HCAPLUS' ENTERED AT 15:58:09 ON 09 AUG 2002

=> s l3 and (vaccin? or ?immun?)
         55731 VACCIN?
        689500 ?IMMUN?
L8          14 L3 AND (VACCIN? OR ?IMMUN?)

=> s l8 not l7
L9          12 L8 NOT L7

=> d stat que
L1              1 SEA FILE=REGISTRY "MSP1 (PROTEIN) (PLASMODIUM VIVAX STRAIN
                  V200 GENE MSP1 C-TERMINAL FRAGMENT)"/CN
L2              1 SEA FILE=REGISTRY IDE8/BI
L3             20 SEA FILE=HCAPLUS L1 OR MSP1A OR MSP1(W)A
L4              7 SEA FILE=HCAPLUS L2 OR IDE8 OR IDE(W)8
L7              2 SEA FILE=HCAPLUS (L3 AND L4) AND (VACCIN? OR ?IMMUN?)
L8             14 SEA FILE=HCAPLUS L3 AND (VACCIN? OR ?IMMUN?)
```

L9 12 SEA FILE=HCAPLUS L8 NOT L7

=> d ibib abs hitrn 19 1-12

L9 ANSWER 1 OF 12 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:282359 HCAPLUS
TITLE: A msp1.alpha. polymerase chain reaction assay for specific detection and differentiation of Anaplasma marginale isolates
AUTHOR(S): Lew, A. E.; Bock, R. E.; Minchin, C. M.; Masaka, S.
CORPORATE SOURCE: Agency for Food and Fibre Sciences, Queensland Department of Primary Industries, c/o Animal Research Institute, Moorooka, 4105, Australia
SOURCE: Veterinary Microbiology (2002), 86(4), 325-335
CODEN: VMICDQ; ISSN: 0378-1135
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Anaplasma marginale is the causative agent of bovine anaplasmosis, a disease which can be protected by **vaccination** with the less pathogenic Anaplasma species, A. centrale. Currently, there is no polymerase chain reaction (PCR) assay available which differentiates between different species of Anaplasma or which can differentiate isolates of A. marginale within outbreaks and between different countries. A mol. test specific for A. marginale would be ideal for the identification of Anaplasma species in wild ruminants, as possible reservoirs of anaplasmosis, and to differentiate between A. marginale from A. centrale. A PCR assay was designed to amplify the major surface protein 1.alpha. gene of the rickettsial bovine pathogen, A. marginale both as an inter- and intra-specific test. The test did not amplify A. centrale or A. ovis, and discriminated A. marginale by amplifying repeat regions within the msp1.alpha. gene which vary in no. between many isolates. The nested A. marginale amplicons varied in size from 630 to 1190 bp representing one to eight internal repeats. All 22 Australian isolates tested amplified a 630 bp product (one repeat) in contrast to all 19 non-Australian isolates tested. Eight sequences from Australian isolates from different geog. regions confirmed the conserved nature of the Australian A. marginale msp1.alpha. genes. The Australian 'repeat unit' **MSP1a** deduced amino acid sequence has been designated as Australian type 1. The msp1.alpha. PCR method developed here enabled the amplification and comparison of A. marginale isolates originating from North and South America, Africa, Israel and Australia. The method is sensitive and specific for A. marginale. Although addnl. msp1.alpha. products were amplified from at least two Australian isolates, the results suggest limited introduction of A. marginale into Australia.
REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 2 OF 12 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:217213 HCAPLUS
TITLE: Evolution and function of tandem repeats in the major surface protein 1a of the ehrlichial pathogen Anaplasma marginale

AUTHOR(S): De la Fuente, Jose; Garcia-Garcia, Jose C.; Blouin, Edmour F.; Rodriguez, Sergio D.; Garcia, Migel A.; Kocan, Katherine M.

CORPORATE SOURCE: Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, 74078, USA

SOURCE: Animal Health Research Reviews (2001), 2(2), 163-173
CODEN: AHRRCJ; ISSN: 1466-2523

PUBLISHER: CABI Publishing
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The major surface protein (MSP) 1a of the ehrlichial cattle pathogen Anaplasma marginale, encoded by the single-copy gene msp1.alpha., has been shown to have a neutralization-sensitive epitope and to be an adhesin for bovine erythrocytes and tick cells. Msp1.alpha. has been found to be a stable genetic marker for the identification of geog. isolates of A. marginale throughout development in acutely and persistently infected cattle and in ticks. The mol. wt. of **MSP1a** varies among geog. isolates of A. marginale because of a varying no. of tandemly repeated peptides of 28-29 amino acids. Variation in the sequence of the tandem repeats occurs within and among isolates, and may have resulted from evolutionary pressures exerted by ligand-receptor and host-parasite interactions. These repeated sequences include markers for tick transmissibility that may be important in the identification of ehrlichial pathogens because they may influence control strategies and the design of subunit **vaccines**.

REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 3 OF 12 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:79033 HCAPLUS
DOCUMENT NUMBER: 137:42389
TITLE: Conservation of major surface protein 1 genes of Anaplasma marginale during cyclic transmission between ticks and cattle

AUTHOR(S): Bowie, Michael V.; de la Fuente, Jose; Kocan, Katherine M.; Blouin, Edmour F.; Barbet, Anthony F.

CORPORATE SOURCE: Department of Pathobiology, University of Florida, Gainesville, FL, 32611-0880, USA

SOURCE: Gene (2002), 282(1-2), 95-102
CODEN: GENED6; ISSN: 0378-1119

PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bovine anaplasmosis is a rickettsial disease of world-wide economic importance caused by Anaplasma marginale. Several major surface proteins with conserved gene sequences have been examd. as potential candidates for **vaccines** and/or diagnostic assays. Major surface protein 1 (MSP1) is composed of polypeptides **MSP1a** and MSP1b. **MSP1a** is expressed from the single copy gene msp1.alpha. and MSP1b is expressed by members of the msp1.beta. multigene family. In order to det. if the msp1 genes are conserved, primers specific for msp1.alpha., msp1.beta.1, and msp1.beta.2 genes were synthesized and used to amplify msp1 sequences of A. marginale from tick cell cultures, from cattle during acute and chronic

infections and from salivary glands of Dermacentor variabilis. Protein sequences of **MSP1a**, MSP1b1 and MSP1b2 were conserved during the life cycle of the parasite. No amino acid changes were obsd. in **MSP1a**. However, small variations were obsd. in the MSP1b1 and MSP1b2 protein sequences, which could be attributed to recombination, selection for sub-populations of A. marginale in the vertebrate host and/or PCR errors. Several isolate-specific sequences were also obsd. Based on the information obtained in this study, the MSP1 protein appears to be fairly well conserved and a potential **vaccine** candidate.

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 4 OF 12 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:10682 HCAPLUS
DOCUMENT NUMBER: 136:81692
TITLE: Human mitochondrial dynamin MSP1 and its isoforms and their role in dominant optical atrophy and therapeutic use
INVENTOR(S): Lenaers, Guy; Ducommun, Bernard; Hamel, Christian; Delettre, Cecile; Belenguer, Pascale
PATENT ASSIGNEE(S): Universite Paul Sabatier, Fr.; Institut National de la Sante et de la Recherche Medicale
SOURCE: PCT Int. Appl., 75 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002000878	A2	20020103	WO 2001-FR1999	20010625
W: US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
FR 2810673	A1	20011228	FR 2000-8140	20000626

PRIORITY APPLN. INFO.: FR 2000-8140 A 20000626

AB The invention concerns a human protein belonging to the family of dynamins, called MSP1, and its 7 MSP1-X isoforms, whereof the mutations are in particular responsible for dominant optical atrophy. The proteins are orthologs of yest msp1 and MGM1 proteins. The invention also concerns nucleotide sequences coding for said proteins, its isoforms and their mutated forms, vectors capable of expressing said protein and its isoforms and their mutated forms, in any type of host cells, and cells transformed by said vectors and methods using them. The invention further concerns methods for identifying biol. or pharmacol. compds. modulating the activity of the inventive protein and its isoforms and the use of said compds. for research and manuf. of active substances useful in therapeutics, in particular for prepg. treatment of dominant optical atrophy. The gene was identified in a public sequence database by its homol. to the Schizosaccharomyces pombe msp1 gene. The gene was expressed in Escherichia coli, S. pombe and HeLa cells.

L9 ANSWER 5 OF 12 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:865935 HCAPLUS
DOCUMENT NUMBER: 136:260948
TITLE: Major surface protein 1a effects tick infection and transmission of Anaplasma marginale
AUTHOR(S): de la Fuente, Jose; Garcia-Garcia, Jose C.; Blouin, Edmour F.; McEwen, Brian R.; Clawson, Dollie; Kocan, Katherine M.
CORPORATE SOURCE: College of Veterinary Medicine, Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, 74078, USA
SOURCE: International Journal for Parasitology (2001), 31(14), 1705-1714
CODEN: IJPYBT; ISSN: 0020-7519
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Anaplasma marginale, an ehrlichial pathogen of cattle and wild ruminants, is transmitted biol. by ticks. A developmental cycle of A. marginale occurs in a tick that begins in gut cells followed by infection of salivary glands, which are the site of transmission to cattle. Geog. isolates of A. marginale vary in their ability to be transmitted by ticks. In these expts. the authors studied transmission of two recent field isolates of A. marginale, an Oklahoma isolate from Wetumka, OK, and a Florida isolate from Okeechobee, FL, by two populations of Dermacentor variabilis males obtained from the same regions. The Florida and Oklahoma tick populations transmitted the Oklahoma isolate, while both tick populations failed to transmit the Florida isolate. Gut and salivary gland infections of A. marginale, as detd. by quant. PCR and microscopy, were detected in ticks exposed to the Oklahoma isolate, while these tissues were not infected in ticks exposed to the Florida isolate. An adhesion-recovery assay was used to study adhesion of the A. marginale major surface protein (MSP) 1a to gut cells from both tick populations and cultured tick cells. The authors demonstrated that recombinant Escherichia coli expressing Oklahoma **MSP1a** adhered to cultured and native D. variabilis gut cells, while recombinant E. coli expressing the Florida **MSP1a** were not adherent to either tick cell population. The **MSP1a** of the Florida isolate of A. marginale, therefore, was unable to mediate attachment to tick gut cells, thus inhibiting salivary gland infection and transmission to cattle. This is the first report of **MSP1a** being responsible for effecting infection and transmission of A. marginale by Dermacentor spp. ticks. The mechanism of tick infection and transmission of A. marginale is important in formulating control strategies and development of improved **vaccines** for anaplasmosis.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 6 OF 12 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:790975 HCAPLUS
DOCUMENT NUMBER: 136:68379
TITLE: CD4+ T lymphocytes from calves **immunized** with Anaplasma marginale major surface protein 1 (**MSP1**), **a** heteromeric complex of **MSP1a** and MSP1b, preferentially recognize the

MSP1a carboxyl terminus that is conserved among strains

AUTHOR(S): Brown, Wendy C.; Palmer, Guy H.; Lewin, Harris A.; McGuire, Travis C.

CORPORATE SOURCE: Program in Vector-Borne Diseases, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, 99164, USA

SOURCE: Infection and Immunity (2001), 69(11), 6853-6862
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Native major surface protein 1 (MSP1) of the ehrlichial pathogen Anaplasma marginale induces protective **immunity** in calves challenged with homologous and heterologous strains. MSP1 is a heteromeric complex of a single **MSP1a** protein covalently assocd. with MSP1b polypeptides, of which at least two (designated MSP1F1 and MSP1F3) in the Florida strain are expressed. **Immunization** with recombinant **MSP1a** and MSP1b alone or in combination fails to provide protection. The protective **immunity** in calves **immunized** with native MSP1 is assocd. with the development of opsonizing and neutralizing antibodies, but CD4+ T-lymphocyte responses have not been evaluated. CD4+ T lymphocytes participate in protective **immunity** to ehrlichial pathogens through prodn. of gamma interferon (IFN-.gamma.), which promotes switching to high-affinity IgG and activation of phagocytic cells to produce nitric oxide. Thus, an effective **vaccine** for A. marginale and related organisms should contain both T- and B-lymphocyte epitopes that induce a strong memory response that can be recalled upon challenge with homologous and heterologous strains. This study was designed to det. the relative contributions of **MSP1a** and MSP1b proteins, which contain both variant and conserved amino acid sequences, in stimulating memory CD4+ T-lymphocyte responses in calves **immunized** with native MSP1. Peripheral blood mononuclear cells and CD4+ T-cell lines from MSP1-**immunized** calves proliferated vigorously in response to the **immunizing** strain (Florida) and heterologous strains of A. marginale. The conserved MSP1-specific response was preferentially directed to the C-terminal region of **MSP1a**, which stimulated high levels of IFN-.gamma. prodn. by CD4+ T cells. In contrast, there was either weak or no recognition of MSP1b proteins. Paradoxically, all calves developed high titers of IgG antibodies to both **MSP1a** and MSP1b polypeptides. These findings suggest that in calves **immunized** with MSP1 heteromeric complex, **MSP1a**-specific T lymphocytes may provide help to MSP1b-specific B lymphocytes. The data provide a basis for detg. whether selected **MSP1a** CD4+ T-lymphocyte epitopes and selected **MSP1a** and MSP1b B-lymphocyte epitopes presented on the same mol. can stimulate a protective **immune** response.

REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 7 OF 12 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:163532 HCAPLUS

DOCUMENT NUMBER: 135:255357

TITLE: Differential adhesion of major surface proteins 1a and

1b of the ehrlichial cattle pathogen Anaplasma marginale to bovine erythrocytes and tick cells

AUTHOR(S): de la Fuente, J.; Garcia-Garcia, J. C.; Blouin, E. F.; Kocan, K. M.

CORPORATE SOURCE: College of Veterinary Medicine, Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, 74078, USA

SOURCE: International Journal for Parasitology (2001), 31(2), 145-153
CODEN: IJPYBT; ISSN: 0020-7519

PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Anaplasma marginale is a tick-borne ehrlichial pathogen of cattle for which six major surface proteins (MSPs) have been described. The MSP1 complex, a heterodimer composed of **MSP1a** and MSP1b, was shown to induce a protective **immune** response in cattle and both proteins have been identified as putative adhesins for bovine erythrocytes. In this study the role of **MSP1a** and MSP1b as adhesins for bovine erythrocytes and tick cells was defined. msp1.alpha. and msp1.beta.1 genes from the Oklahoma isolate of A. marginale were cloned and expressed in Escherichia coli K-12 under the control of endogenous and tac promoters for both low and high level protein expression. Expression of the recombinant polypeptides was confirmed and localized on the surface of transformed E. coli. The adhesion properties of **MSP1a** and MSP1b were detd. by allowing recombinant E. coli expressing these surface polypeptides to react with bovine erythrocytes, Dermacentor variabilis gut cells and cultured tick cells derived from embryonic Ixodes scapularis. Adhesion of the recombinant E. coli to the three cell types was detd. using recovery adhesion and microtiter hemagglutination assays, and by light and electron microscopy. **MSP1a** was shown by all methods tested to be an adhesin for bovine erythrocytes and both native and cultured tick cells. In contrast, recombinant E. coli expressing MSP1b adhered only to bovine erythrocytes and not to tick cells. When low expression vectors were used, single E. coli expressing **MSP1a** was seen adhered to individual tick cells while reaction of tick cells with the E. coli/**MSP1a**/high expression vector resulted in adhesion of multiple bacteria per cell. With electron microscopy, fusion of E. coli cell membranes expressing **MSP1a** or MSP1b with erythrocyte membranes was obsd., as well as fusion of tick cell membranes with E. coli membranes expressing **MSP1a**. These studies demonstrated differential adhesion for **MSP1a** and MSP1b for which **MSP1a** is an A. marginale adhesin for both bovine erythrocytes and tick cells while MSP1b is an adhesin only for bovine erythrocytes. The role of the MSP1 complex, therefore, appears to vary among vertebrate and invertebrate hosts.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 8 OF 12 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:589704 HCAPLUS
DOCUMENT NUMBER: 134:52061
TITLE: Intragenic recombination in the 3' portion of the merozoite surface protein 1 gene of Plasmodium vivax

AUTHOR(S): Putaporntip, C.; Jongwutiwes, S.; Seethamchai, S.; Kanbara, H.; Tanabe, K.
CORPORATE SOURCE: Faculty of Medicine, Department of Parasitology, Chulalongkorn University, Bangkok, 10330, Thailand
SOURCE: Molecular and Biochemical Parasitology (2000), 109(2), 111-119
CODEN: MBIPDP; ISSN: 0166-6851
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To date, little has been known about the extent of sequence variation in the C-terminal part of the Plasmodium vivax merozoite surface protein 1 (PvMSP1) which has been considered to be a potential **vaccine** candidate. Here, the authors examd. the variation in the region encompassing interspecies conserved blocks (ICBs) 8 and 10 of PvMSP1 by DNA sequencing of 14 Thai isolates and three Brazilian isolates. Eighteen different alleles were detected. Three new sequence types had been identified in polymorphic region between ICB8 and CB9: one was possibly a result of intragenic recombination between the Belem and Salvador I alleles and the others displayed unique repeats. A striking variation was obsd. in a stretch of 38 codons in polymorphic block between conserved block CB9 and ICB10, resulting in eight different sequence types, probably generated by interallelic recombination at a single or multiple sites. There is no apparent linkage between these two polymorphic sites. On the other hand, a single or stretches of nucleotide substitutions are dimorphic like in Plasmodium falciparum MSP1 (PfMSP1) in the remaining parts, creating microheterogeneity of sequences. The C-terminal 19 kDa-encoding region was extremely conserved with a single dimorphic exchange at a known position. Thus, this study provides evidence of intragenic recombination occurring in the 3' portion of PvMSP1 and suggests that the 3' portion of PvMSP1 is more diverse than that in PfMSP1.

IT **313406-46-1**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; intragenic recombination in the 3' portion of the merozoite surface protein 1 gene of Plasmodium vivax)

L9 ANSWER 9 OF 12 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:213574 HCAPLUS
DOCUMENT NUMBER: 132:346075
TITLE: Expression of polymorphic msp1.beta. genes during acute Anaplasma marginale rickettsemia
AUTHOR(S): Camacho-Nuez, Minerva; Munoz, Maria de Lourdes; Suarez, Carlos E.; McGuire, Travis C.; Brown, Wendy C.; Palmer, Guy H.
CORPORATE SOURCE: Departamento de Genetica y Biologia Molecular, CINVESTAV-IPN, Mexico, 07000, Mex.
SOURCE: Infection and Immunity (2000), 68(4), 1946-1952
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Immunization** of cattle with native MSP1 induces protection

against Anaplasma marginale. The native **immunogen** is composed of a single **MSP1a** protein and multiple, undefined MSP1b polypeptides. In addn. to the originally sequenced gene, designated msp1.beta.(F1), we identified three complete msp1.beta. genes in the Florida strain: msp1.beta.(F2), msp1.beta.(F3), and msp1.beta.(F4). Each of these polymorphic genes encodes a structurally unique MSP1b protein, and unique transcripts can be identified during acute A. marginale rickettsemia. The structural polymorphism is clustered in discrete variable regions, and each MSP1b protein results from a unique mosaic of five variable regions. Although each of the MSP1b proteins in the Florida strain contains epitopes recognized by serum antibody induced by protective **immunization** with the native MSP1 complex, the variable regions also include epitopes expressed by some but not all of the MSP1b proteins. These data support testing recombinant **vaccines** composed of the multiple antigenically and structurally unique MSP1b proteins combined with **MSP1a** in order to mimic the efficacy of native MSP1 **immunization**.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 10 OF 12 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:412236 HCAPLUS
DOCUMENT NUMBER: 131:198351
TITLE: Biased **immunoglobulin** G1 isotype responses induced in cattle with DNA expressing **msp1a** of Anaplasma marginale
AUTHOR(S): Arulkanthan, Appudurai; Brown, Wendy C.; McGuire, Travis C.; Knowles, Donald P.
CORPORATE SOURCE: Program in Vector-Borne Diseases, Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA, 99164, USA
SOURCE: Infection and Immunity (1999), 67(7), 3481-3487
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Immunization** with the native major surface protein 1 (**MSP1**) (**a** heterodimer contg. disulfide and noncovalently bonded polypeptides designated **MSP1a** and MSP1b) of the erythrocytic stage of Anaplasma marginale conferred protection against homologous challenge. The **MSP1a** polypeptide possesses a conserved neutralization-sensitive epitope. In the present study, the **immune** response to DNA-mediated **immunization** using **msp1a** was studied. The plasmid pVCL/**MSP1a**, which encodes the complete **msp1a** gene of A. marginale under the control of human cytomegalovirus immediate-early enhancer/promoter and intron A, was constructed. The **immune** responses elicited by **immunization** with pVCL/**MSP1a** into cardiotoxin-induced regenerating muscle were evaluated in mice and cattle. Antibody reactive with native **MSP1a** was detected in pooled sera of **immunized** BALB/c mice 3 wk following primary **immunization** . Two calves seroneg. for A. marginale were **immunized** four times, at weeks 0, 3, 7, and 13, with pVCL/**MSP1a**. By 8 wk, both

calves responded to **MSP1a** with an antibody titer of 1:100, which peaked at 1:1600 and 1:800 by 16 wk after the initial **immunization**. Interestingly, **immunoblotting** with anti-IgG1 and anti-IgG2 specific monoclonal antibodies revealed a restricted IgG1 anti-**MSP1a** response in both animals. T-lymphocyte lines, established after the fourth **immunization**, proliferated specifically against A. marginale homogenate and purified MSP1 in a dose-dependent manner. These data provide a basis for an **immunization** strategy to direct bovine **immune** responses by using DNA **vaccine** vectors contg. single or multiple genes encoding major surface proteins of A. marginale.

REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 11 OF 12 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:11262 HCAPLUS
DOCUMENT NUMBER: 122:29569
TITLE: Recombinant **vaccinia** virus expression of Anaplasma marginale surface protein MSP-1a: Effect of promoters, leader sequences and GPI anchor sequence on antibody response
AUTHOR(S): McGuire, Travis C.; Stephens, Edward B.; Palmer, Guy H.; McElwain, Terry F.; Lichtensteiger, Carol A.; Leib, Steve R.; Barbet, Anthony F.
CORPORATE SOURCE: Coll. Vet. Med., Wash. State Univ., Pullman, WA, 99164-7040, USA
SOURCE: Vaccine (1994), 12(5), 465-72
CODEN: VACCDE; ISSN: 0264-410X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Anaplasma marginale surface protein MSP-1a was expressed by recombinant **vaccinia** viruses with different promoters and as hybrid proteins. Transcription of MSP-1a with P11 late promoter resulted in more MSP-1a than with P7.5 early-late promoter; however, mice **immunized** with the recombinants had similar antibody titers. Recombinants expressing hybrid MSP-1a with either a murine leukemia virus or a trypanosomal glycoprotein signal sequence did not enhance antibody responses and resulted in a diffuse intracellular distribution of MSP-1a which did not accumulate in the Golgi app. as was noted in the absence of these signal sequences. In contrast, antibody titers to MSP-1a in mice **immunized** with a recombinant virus expressing hybrid MSP-1a with a trypanosomal GPI anchor signal sequence were significantly increased over all other constructs.

L9 ANSWER 12 OF 12 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:696732 HCAPLUS
DOCUMENT NUMBER: 121:296732
TITLE: Putative adhesins of Anaplasma marginale: major surface polypeptides 1a and 1b
AUTHOR(S): McGarey, Donald J.; Barbet, Anthony F.; Palmer, Guy H.; McGuire, Travis C.; Allred, David R.
CORPORATE SOURCE: Department of Infectious Diseases, University of Florida, Gainesville, FL, 32611, USA
SOURCE: Infect. Immun. (1994), 62(10), 4594-601

CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Genes for the **MSP1a** and MSP1b subunits of the Anaplasma marginale surface antigen MSP1 were previously cloned and expressed in Escherichia coli. The authors report here the localization of **MSP1a** and MSP1b polypeptides on the surface of recombinant E. coli by using a live cell indirect **immunofluorescent** antibody assay. Recombinant E. coli cells expressing the msp1.alpha. gene or the msp1.beta. gene encoding the **MSP1a** and MSP1b polypeptide subunits, resp., were shown by a culture recovery adhesion assay and by direct microscopic examn. to specifically adhere to bovine erythrocytes. This adhesion was more than additive when both genes were coexpressed in a single recombinant construct. Similarly, these recombinants hemagglutinated bovine erythrocytes in a microtiter hemagglutination assay. Inhibition of recombinant E. coli adhesion to bovine erythrocytes and hemagglutination inhibition were obsd. in the presence of homologous monospecific polyclonal antiserum raised against purified **MSP1a** or MSP1b polypeptide. These data suggest that the **MSP1a** and MSP1b polypeptides have functions as adhesins on A. marginale initial bodies, probably during erythrocyte invasion.

=> d stat que
L1 1 SEA FILE=REGISTRY "MSP1 (PROTEIN) (PLASMODIUM VIVAX STRAIN V200 GENE MSP1 C-TERMINAL FRAGMENT)"/CN
L2 1 SEA FILE=REGISTRY IDE8/BI
L3 20 SEA FILE=HCAPLUS L1 OR MSP1A OR MSP1(W)A
L4 7 SEA FILE=HCAPLUS L2 OR IDE8 OR IDE(W)8
L8 14 SEA FILE=HCAPLUS L3 AND (VACCIN? OR ?IMMUN?)
L10 3 SEA FILE=HCAPLUS L4 (L)TICK? AND (ANTIGEN? OR AG) AND (VACCIN? OR ?IMMUN?)
L11 2 SEA FILE=HCAPLUS L10 NOT L8

=> d ibib abs hitrn l11 1-2

L11 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:468011 HCAPLUS
DOCUMENT NUMBER: 131:101255
TITLE: In vitro production of Ehrlichia phagocytophila **antigen** in **IDE8 tick** cell line and HL60 cells for diagnosing ehrlichiosis
INVENTOR(S): Dumler, J. Stephen; Munderloh, Ulrike G.; Madigan, John; Goodman, Jesse; Kurtti, Timothy J.
PATENT ASSIGNEE(S): Regents of the University of Minnesota, USA; Regents of the University of California; University of Maryland at Baltimore
SOURCE: U.S., 27 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5928879	A	19990727	US 1995-519283	19950825
US 5955359	A	19990921	US 1997-788711	19970123

PRIORITY APPLN. INFO.: US 1995-519283 19950825

AB Methods for the in vitro cultivation, propagation, and prodn. of **antigens** of Ehrlichia phagocytophila genogroup granulocytic Ehrlichiae, including Ehrlichia equi, in Ixodes scapularis tick cell culture and in human HL60 promyelocytic leukemia cell culture are presented. Establishment, maintenance and description of cell lines from Ixodes scapularis along with the methods for cryopreservation, karyotyping, isoelec. focusing of its enzymes are described. Results of the infection of the tick cell lines with Ehrlichia equi and the infectivity of horses with the pathogens are discussed. Antibody reactivity and the infectivity of human HL60 cells with Ehrlichia equi grown in the tick cell lines is studied.

REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:276425 HCAPLUS
DOCUMENT NUMBER: 126:248588
TITLE: Method of growing rickettsiae in Ixodes scapularis tick cell culture and preparing **antigens** and **vaccines** of rickettsiae
INVENTOR(S): Munderloh, Ulrike G.; Kurtti, Timothy J.; Kocan, Katherine M.; Blouin, Edmour F.; Ewing, Sidney A.
PATENT ASSIGNEE(S): Regents of the University of Minnesota, USA; Oklahoma State University
SOURCE: PCT Int. Appl., 89 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9708296	A1	19970306	WO 1996-US13594	19960823
W: AL, AM, AT, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5869335	A	19990209	US 1995-519599	19950825
AU 9668559	A1	19970319	AU 1996-68559	19960823
BR 9610681	A	19990703	BR 1996-10681	19960823

PRIORITY APPLN. INFO.: US 1995-519599 19950825
WO 1996-US13594 19960823

AB The methods of the invention provide for culture of microorganisms such as Anaplasma marginale, Ehrlichia canis, and Rickettsia rickettsii. A method

of the invention involves incubating a rickettsia with an I. scapularis **tick** cell culture in a culture medium under reduced O and increased CO2 at a sufficient temp. until growth of the rickettsia is detected. The culture medium comprises a medium suitable for the growth of invertebrate cells supplemented with an org. buffer. The cell culture method can be used in large-scale prodn. of rickettsia contg. products useful in diagnostic assays and **vaccine** prepns. In one example, A. marginale, which causes anaplasmosis in cattle, was grown in I. scapularis cell culture, and then **antigens** were prepd. for use in **vaccine** prepn. and for diagnostic assays. In other examples, R. rickettsii was grown in **IDE8 tick** cell line culture to study the growth of the spotted fever group of rickettsia and E. canis was propagated in **IDE8 tick** cell culture.

```
=> fil hcaplu
FILE 'HCAPLUS' ENTERED AT 14:19:59 ON 09 AUG 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)
```



```
FILE COVERS 1907 - 9 Aug 2002  VOL 137 ISS 7
FILE LAST UPDATED: 8 Aug 2002  (20020808/ED)

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

CAS roles have been modified effective December 16, 2001. Please
check your SDI profiles to see if they need to be revised. For
information on CAS roles, enter HELP ROLES at an arrow prompt or use
the CAS Roles thesaurus (/RL field) in this file.

=> d stat que
L1            96 SEA FILE=HCAPLUS "DE LA FUENTE J"/AU OR ("DE LA FUENTE
                 JOSE"/AU OR "DE LA FUENTE JOSE DE JESUS"/AU OR "DE LA FUENTE
                 JOSE DE JESUS"/IN)
L2            34 SEA FILE=HCAPLUS ((KOCAN K?) OR (KOCAN,K?) OR (KOCAN, K?))/AU,I
                 N
L3            18 SEA FILE=HCAPLUS ((BLOUIN E?) OR (BLOUIN,E?) OR (BLOUIN,
                 E?))/AU,IN
L4           121 SEA FILE=HCAPLUS L1 OR L2 OR L3 AND (MSPIA OR MARGINALE? OR
                 IDE8)
L6            32 SEA FILE=HCAPLUS L4 AND (IMMUN? OR VACCIN?)


=> d ibib abs hitrn l6 1-32

L6   ANSWER 1 OF 32  HCAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:         2002:353311  HCAPLUS
DOCUMENT NUMBER:          136:368445
TITLE:                    Recombinant major surface protein from Anaplasma
                          marginale for vaccination
INVENTOR(S):              De La Fuente, Jose De Jesus; Kocan,
                          Katherine M.; Garcia-Garcia, Jose Carlos; Blouin,
                          Edmour F.
PATENT ASSIGNEE(S):       The Board of Regents for Oklahoma State University,
                          USA
SOURCE:                   PCT Int. Appl., 30 pp.
```

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002036159	A2	20020510	WO 2001-US48505	20011030

W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2000-244333P P 20001030

AB The authors disclose a **vaccine** prepn. for eliciting an enhanced **immune** response against Anaplasma **marginale**. The **vaccine** comprises recombinant MSP1a surface protein alone or in combination with tick cell culture-derived A. **marginale**.

L6 ANSWER 2 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:217213 HCAPLUS
TITLE: Evolution and function of tandem repeats in the major surface protein 1a of the ehrlichial pathogen Anaplasma **marginale**
AUTHOR(S): **De la Fuente, Jose**; Garcia-Garcia, Jose C.; **Blouin, Edmour F.**; Rodriguez, Sergio D.; Garcia, Migel A.; **Kocan, Katherine M.**
CORPORATE SOURCE: Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, 74078, USA
SOURCE: Animal Health Research Reviews (2001), 2(2), 163-173
CODEN: AHRRCJ; ISSN: 1466-2523
PUBLISHER: CABI Publishing
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The major surface protein (MSP) 1a of the ehrlichial cattle pathogen Anaplasma **marginale**, encoded by the single-copy gene msp1.alpha., has been shown to have a neutralization-sensitive epitope and to be an adhesin for bovine erythrocytes and tick cells. Msp1.alpha. has been found to be a stable genetic marker for the identification of geog. isolates of A. **marginale** throughout development in acutely and persistently infected cattle and in ticks. The mol. wt. of MSP1a varies among geog. isolates of A. **marginale** because of a varying no. of tandemly repeated peptides of 28-29 amino acids. Variation in the sequence of the tandem repeats occurs within and among isolates, and may have resulted from evolutionary pressures exerted by ligand-receptor and host-parasite interactions. These repeated sequences include markers for tick transmissibility that may be important in the identification of

ehrlichial pathogens because they may influence control strategies and the design of subunit **vaccines**.

REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:79033 HCAPLUS
DOCUMENT NUMBER: 137:42389
TITLE: Conservation of major surface protein 1 genes of Anaplasma **marginale** during cyclic transmission between ticks and cattle
AUTHOR(S): Bowie, Michael V.; **de la Fuente, Jose**; **Kocan, Katherine M.**; **Blouin, Edmour F.**; Barbet, Anthony F.
CORPORATE SOURCE: Department of Pathobiology, University of Florida, Gainesville, FL, 32611-0880, USA
SOURCE: Gene (2002), 282(1-2), 95-102
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Bovine anaplasmosis is a rickettsial disease of world-wide economic importance caused by Anaplasma **marginale**. Several major surface proteins with conserved gene sequences have been examd. as potential candidates for **vaccines** and/or diagnostic assays. Major surface protein 1 (MSP1) is composed of polypeptides MSP1a and MSP1b. MSP1a is expressed from the single copy gene msp1.alpha. and MSP1b is expressed by members of the msp1.beta. multigene family. In order to det. if the msp1 genes are conserved, primers specific for msp1.alpha., msp1.beta.1, and msp1.beta.2 genes were synthesized and used to amplify msp1 sequences of A. **marginale** from tick cell cultures, from cattle during acute and chronic infections and from salivary glands of Dermacentor variabilis. Protein sequences of MSP1a, MSP1b1 and MSP1b2 were conserved during the life cycle of the parasite. No amino acid changes were obsd. in MSP1a. However, small variations were obsd. in the MSP1b1 and MSP1b2 protein sequences, which could be attributed to recombination, selection for sub-populations of A. **marginale** in the vertebrate host and/or PCR errors. Several isolate-specific sequences were also obsd. Based on the information obtained in this study, the MSP1 protein appears to be fairly well conserved and a potential **vaccine** candidate.
REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:865935 HCAPLUS
DOCUMENT NUMBER: 136:260948
TITLE: Major surface protein 1a effects tick infection and transmission of Anaplasma **marginale**
AUTHOR(S): **de la Fuente, Jose**; Garcia-Garcia, Jose C.; **Blouin, Edmour F.**; McEwen, Brian R.; Clawson, Dollie; **Kocan, Katherine M.**
CORPORATE SOURCE: College of Veterinary Medicine, Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, 74078, USA

SOURCE: International Journal for Parasitology (2001), 31(14), 1705-1714
CODEN: IJPYBT; ISSN: 0020-7519
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Anaplasma **marginale**, an ehrlichial pathogen of cattle and wild ruminants, is transmitted biol. by ticks. A developmental cycle of A. **marginale** occurs in a tick that begins in gut cells followed by infection of salivary glands, which are the site of transmission to cattle. Geog. isolates of A. **marginale** vary in their ability to be transmitted by ticks. In these expts. the authors studied transmission of two recent field isolates of A. **marginale**, an Oklahoma isolate from Wetumka, OK, and a Florida isolate from Okeechobee, FL, by two populations of Dermacentor variabilis males obtained from the same regions. The Florida and Oklahoma tick populations transmitted the Oklahoma isolate, while both tick populations failed to transmit the Florida isolate. Gut and salivary gland infections of A. **marginale**, as detd. by quant. PCR and microscopy, were detected in ticks exposed to the Oklahoma isolate, while these tissues were not infected in ticks exposed to the Florida isolate. An adhesion-recovery assay was used to study adhesion of the A. **marginale** major surface protein (MSP) 1a to gut cells from both tick populations and cultured tick cells. The authors demonstrated that recombinant Escherichia coli expressing Oklahoma MSP1a adhered to cultured and native D. variabilis gut cells, while recombinant E. coli expressing the Florida MSP1a were not adherent to either tick cell population. The MSP1a of the Florida isolate of A. **marginale**, therefore, was unable to mediate attachment to tick gut cells, thus inhibiting salivary gland infection and transmission to cattle. This is the first report of MSP1a being responsible for effecting infection and transmission of A. **marginale** by Dermacentor spp. ticks. The mechanism of tick infection and transmission of A. **marginale** is important in formulating control strategies and development of improved **vaccines** for anaplasmosis.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 32 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:549234 HCAPLUS
DOCUMENT NUMBER: 135:254742

TITLE: Expression of Anaplasma marginale major surface protein 2 variants in persistently infected ticks

AUTHOR(S): **De la Fuente, Jose; Kocan, Katherine M.**
CORPORATE SOURCE: Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, 74078-2007, USA

SOURCE: Infection and Immunity (2001), 69(8), 5151-5156
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A. marginale, an intraerythrocytic ehrlichial pathogen of cattle,

establishes persistent infections in both vertebrate (cattle) and invertebrate (tick) hosts. The ability of A. marginale to persist in cattle has been shown to be due, in part, to major surface protein 2 (MSP2) variants which are hypothesized to emerge in response to the bovine **immune** response. MSP2 antigenic variation has not been studied in persistently infected ticks. In this study we analyzed MSP2 in A. marginale populations from the salivary glands of male Dermacentor variabilis persistently infected with A. marginale after feeding successively on 1 susceptible bovine and 3 sheep. New MSP2 variants appeared in each A. marginale population, and sequence alignment of the MSP2 variants revealed multiple amino acid substitutions, insertions, and deletions. These results suggest that selection pressure on MSP2 occurred in tick salivary glands independent of the bovine **immune** response.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 6 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:309905 HCAPLUS
DOCUMENT NUMBER: 135:91406
TITLE: Antigenic variation of Anaplasma **marginale**: major surface protein 2 diversity during cyclic transmission between ticks and cattle
AUTHOR(S): Barbet, A. F.; Yi, Jooyoung; Lundgren, Anna; McEwen, B. R.; **Blouin, E. F.; Kocan, K. M.**
CORPORATE SOURCE: Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32611, USA
SOURCE: Infection and Immunity (2001), 69(5), 3057-3066
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The rickettsial pathogen Anaplasma **marginale** expresses a variable **immunodominant** outer membrane protein, major surface protein 2 (MSP2), involved in antigenic variation and long-term persistence of the organism in carrier animals. MSP2 contains a central hypervariable region of about 100 amino acids that encodes **immunogenic** B-cell epitopes that induce variant-specific antibodies during infection. Previously, we have shown that MSP2 is encoded on a polycistronic mRNA transcript in erythrocyte stages of A. **marginale** and defined the structure of the genomic expression site for this transcript. In this study, we show that the same expression site is utilized in stages of A. **marginale** infecting tick salivary glands. We also analyzed the variability of this genomic expression site in Oklahoma strain A. **marginale** transmitted from in vitro cultures to cattle and between cattle and ticks. The structure of the expression site and flanking regions was conserved except for sequence that encoded the MSP2 hypervariable region. At least three different MSP2 variants were encoded in each A. **marginale** population. The major sequence variants did not change on passage of A. **marginale** between culture, acute erythrocyte stage infections, and tick salivary glands but did change during persistent infections of cattle. The variant types found in tick salivary glands most closely resembled those present

in bovine blood at the time of acquisition of infection, whether infection was acquired from an acute or from a persistent rickettsemia. These variations in structure of an expression site for a major, **immunoprotective** outer membrane protein have important implications for **vaccine** development and for obtaining an improved understanding of the mechanisms of persistence of ehrlichial infections in humans, domestic animals, and reservoir hosts.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 7 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:163532 HCAPLUS
DOCUMENT NUMBER: 135:255357
TITLE: Differential adhesion of major surface proteins 1a and 1b of the ehrlichial cattle pathogen Anaplasma **marginale** to bovine erythrocytes and tick cells
AUTHOR(S): **de la Fuente, J.**; Garcia-Garcia, J. C.; **Blouin, E. F.**; **Kocan, K. M.**
CORPORATE SOURCE: College of Veterinary Medicine, Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, 74078, USA
SOURCE: International Journal for Parasitology (2001), 31(2), 145-153
CODEN: IJPYBT; ISSN: 0020-7519
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Anaplasma **marginale** is a tick-borne ehrlichial pathogen of cattle for which six major surface proteins (MSPs) have been described. The MSP1 complex, a heterodimer composed of MSP1a and MSP1b, was shown to induce a protective **immune** response in cattle and both proteins have been identified as putative adhesins for bovine erythrocytes. In this study the role of MSP1a and MSP1b as adhesins for bovine erythrocytes and tick cells was defined. msp1.alpha. and msp1.beta.1 genes from the Oklahoma isolate of A. **marginale** were cloned and expressed in Escherichia coli K-12 under the control of endogenous and tac promoters for both low and high level protein expression. Expression of the recombinant polypeptides was confirmed and localized on the surface of transformed E. coli. The adhesion properties of MSP1a and MSP1b were detd. by allowing recombinant E. coli expressing these surface polypeptides to react with bovine erythrocytes, Dermacentor variabilis gut cells and cultured tick cells derived from embryonic Ixodes scapularis. Adhesion of the recombinant E. coli to the three cell types was detd. using recovery adhesion and microtiter hemagglutination assays, and by light and electron microscopy. MSP1a was shown by all methods tested to be an adhesin for bovine erythrocytes and both native and cultured tick cells. In contrast, recombinant E. coli expressing MSP1b adhered only to bovine erythrocytes and not to tick cells. When low expression vectors were used, single E. coli expressing MSP1a was seen adhered to individual tick cells while reaction of tick cells with the E. coli/MSP1a/high expression vector resulted in adhesion of multiple bacteria per cell. With electron microscopy, fusion of E. coli cell membranes expressing MSP1a or MSP1b with erythrocyte membranes was obsd., as well as fusion of

tick cell membranes with E. coli membranes expressing MSP1a. These studies demonstrated differential adhesion for MSP1a and MSP1b for which MSP1a is an A. **marginale** adhesin for both bovine erythrocytes and tick cells while MSP1b is an adhesin only for bovine erythrocytes. The role of the MSP1 complex, therefore, appears to vary among vertebrate and invertebrate hosts.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 8 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:72223 HCAPLUS
DOCUMENT NUMBER: 135:179172
TITLE: **Immunological** control of ticks through **vaccination** with Boophilus microplus gut antigens
AUTHOR(S): **De La Fuente, Jose**; Rodriguez, Manuel; Garcia-Garcia, Jose C.
CORPORATE SOURCE: Mammalian Cell Genetics Division, Centro de Ingenieria Genetica y Biotecnologia, Havana, Cuba
SOURCE: Annals of the New York Academy of Sciences (2000), 916(Tropical Veterinary Diseases), 617-621
CODEN: ANYAA9; ISSN: 0077-8923
PUBLISHER: New York Academy of Sciences
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 8 refs. The control of tick infestations and the transmission of tick-borne diseases remain a challenge for the scientific community. Traditional control methods have been only partially successful. Recently, **vaccination** with recombinant Boophilus microplus gut antigens has been shown to control tick infestations. Our Bm86-contg. **vaccine** formulation (Gavac) has been effective for the control of artificial infestations of B. annulatus, B. decoloratus, and chem. sensitive and resistant B. microplus strains from Australia, Africa, America, and Iran. Preliminary results with Hyalomma spp. and Rhipicephalus spp. suggest partial cross protection. In field trials, **vaccination** with Gavac controlled B. microplus and B. annulatus infestations and reduced the transmission of babesiosis, resulting in important savings for the cattle industry. Different degrees of susceptibility to the **vaccination** with Bm86 and sequence variations in the Bm86 locus have been reported. The Bm95 antigen was isolated from the Argentinean Bm86-resistant B. microplus strain A. A Bm95-based **vaccine** was used to protect cattle against tick infestations under prodn. conditions with similar results to that obtained with Gavac. The Bm95 antigen from strain A was able to protect against infestations with Bm86-sensitive and Bm86-resistant tick strains, thus suggesting that Bm95 could be a more universal antigen in protecting cattle against infestations by B. microplus strains from different geog. areas. These results clearly demonstrate the advantage and possibilities for the **immunol**. control of ticks.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 9 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:355303 HCAPLUS

DOCUMENT NUMBER: 134:3831
TITLE: Control of ticks resistant to **immunization** with Bm86 in cattle **vaccinated** with the recombinant antigen Bm95 isolated from the cattle tick, Boophilus microplus
AUTHOR(S): Garcia-Garcia, Jose C.; Montero, Carlos; Redondo, Miguel; Vargas, Milagros; Canales, Mario; Boue, Oscar; Rodriguez, Manuel; Joglar, Marisdania; Machado, Hector; Gonzalez, Iliana L.; Valdes, Mario; Mendez, Luis; **De la Fuente, Jose**
CORPORATE SOURCE: Mammalian Cell Genetics Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba
SOURCE: Vaccine (2000), 18(21), 2275-2287
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The recombinant Bm86-contg. **vaccine** Gavac against the cattle tick Boophilus microplus has proved its efficacy in a no. of expts., esp. when combined with acaricides in an integrated manner. However, tick isolates such as the Argentinean strain A, show low susceptibility to this **vaccine**. In this paper we report on the isolation of the Bm95 gene from the B. microplus strain A, which was cloned and expressed in the yeast Pichia pastoris producing a glycosylated and particulated recombinant protein. This new antigen was effective against different tick strains in a pen trial, including the B. microplus strain A, resistant to **vaccination** with Bm86. A Bm95-based **vaccine** was used to protect cattle against tick infestations under prodn. conditions, lowering the no. of ticks on **vaccinated** animals and, therefore, reducing the frequency of acaricide treatments. The Bm95 antigen from strain A was able to protect against infestations with Bm86-sensitive and Bm86-resistant tick strains, thus suggesting that Bm95 could be a more universal antigen to protect cattle against infestations by B. microplus strains from different geog. areas.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 10 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:61449 HCAPLUS
DOCUMENT NUMBER: 132:249709
TITLE: Sequence variations in the Boophilus microplus Bm86 locus and implications for **immunoprotection** in cattle **vaccinated** with this antigen
AUTHOR(S): Garcia-Garcia, Jose C.; Gonzalez, Ileana L.; Gonzalez, Diana M.; Valdes, Mario; Mendez, Luis; Lamberti, Jorge; D'Agostino, Beatriz; Citroni, Daniel; Fragoso, Hugo; Ortiz, Martin; Rodriguez, Manuel; **De La Fuente, Jose**
CORPORATE SOURCE: Mammalian Cell Genetics Division, Centro de Ingenieria Genetica y Biotecnologia, Havana, Cuba
SOURCE: Experimental and Applied Acarology (1999), 23(11), 883-895
CODEN: EAACEM; ISSN: 0168-8162
PUBLISHER: Kluwer Academic Publishers

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Cattle tick infestations constitute a major problem for the cattle industry in tropical and subtropical regions of the world. Traditional control methods have been only partially successful, hampered by the selection of chem.-resistant tick populations. The Boophilus microplus Bm86 protein was isolated from tick gut epithelial cells and shown to induce a protective response against tick infestations in **vaccinated** cattle. **Vaccine** prepns. including the recombinant Bm86 are used to control cattle tick infestations in the field as an alternative measure to reduce the losses produced by this ectoparasite. The principle for the **immunol.** control of tick infestations relies on a polyclonal antibody response against the target antigen and, therefore, should be difficult to select for tick-resistant populations. However, sequence variations in the Bm86 locus, among other factors, could affect the effectiveness of Bm86-contg. **vaccines**. In the present study we have addressed this issue, employing data obtained with B. microplus strains from Australia, Mexico, Cuba, Argentina and Venezuela. The results showed a tendency in the inverse correlation between the efficacy of the **vaccination** with Bm86 and the sequence variations in the Bm86 locus (R2 = 0.7). The mutation fixation index in the Bm86 locus was calcd. and shown to be between 0.02 and 0.1 amino acids per yr. Possible implications of these findings for the **immunoprotection** of cattle against tick infestations employing the Bm86 antigen are discussed.
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 11 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:803889 HCAPLUS
DOCUMENT NUMBER: 132:74861
TITLE: Integrated control of acaricide-resistant Boophilus microplus populations on grazing cattle in Mexico using **vaccination** with Gavac and amidine treatments
AUTHOR(S): Redondo, Miguel; Fragoso, Hugo; Ortiz, Martin; Montero, Carlos; Lona, Julian; Medellin, Jose Antonio; Fria, Ramiro; Hernandez, Victor; Franco, Ruben; Machado, Hector; Rodriguez, Manuel; **De la Fuente, Jose**
CORPORATE SOURCE: Mammalian Cells Genetics Division, Centro de Ingenieria Genetica Biotecnologia, Havana, Cuba
SOURCE: Experimental and Applied Acarology (1999), 23(10), 841-849
CODEN: EAACEM; ISSN: 0168-8162
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Throughout most of the twentieth century, tick infestations on cattle have been controlled with chem. acaricides, typically administered by dipping or spraying. This approach can cause environmental and residue problems and has created a high incidence of acaricide resistance within tick populations in the field. Recently we developed a **vaccine** against Boophilus microplus employing a recombinant Bm86 antigen prepn.

(Gavac), which has been shown to induce a protective response in **vaccinated** animals. Under field conditions, a near 100% control of B. microplus populations resistant to pyrethroids and organophosphates was achieved, by an integrated system employing **vaccination** with Gavac and amidine treatments. This method controls tick infestations while reducing the no. of chem. acaricide treatments and consequently the rise of B. microplus populations resistant to chem. acaricides.

REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 12 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:746488 HCAPLUS
DOCUMENT NUMBER: 132:231698
TITLE: A mutant streptokinase lacking the C-terminal 42 amino acids is less **immunogenic**
AUTHOR(S): Torrens, I.; Ojalvo, A. G.; Seralena, A.; Hayes, O.; **de la Fuente, J.**
CORPORATE SOURCE: Centro de Ingenieria Genetica y Biotecnologia, Division of Pharmaceutical, Havana, Cuba
SOURCE: Immunology Letters (1999), 70(3), 213-218
CODEN: IMLED6; ISSN: 0165-2478
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Streptokinase (SK) is the most widely used compd. for the treatment of myocardial infarction and the least expensive thrombolytic agent, but a drawback to its use is the widespread presence of anti-SK antibodies (Abs). Clin. failure of the activation of the fibrinolytic system by SK has been reported due to the presence of a high titer of anti-SK neutralizing Abs. Patients receiving SK therapy develop high anti-SK antibody titers, which might provoke severe allergic reactions. These Abs are sufficient to neutralize a std. dose of SK up to four years after initial SK administration. This is a clin. problem because of the increasing no. of patients who have been treated once with SK for acute myocardial infarction (AMI) and are likely to require plasminogen activator treatment in the future. In previous in vitro studies, we have shown that a deletion mutant (mut-C42), lacking the 42 C-terminal residues, was significantly less antigenic when compared with the native mol. (SKC-2). In this study, 14 monkeys were subjected to treatment with SKC-2 and mut-C42 in order to compare their humoral response by detg. SK neutralizing activity in monkey's sera. All monkeys developed anti-SKC-2 Ab titers, but in the case where treatment induced Abs directed against the C-terminus of SKC-2, neutralizing activity against the native protein was significantly higher than that developed against mutant SK mut-C42.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 13 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:733418 HCAPLUS
DOCUMENT NUMBER: 132:92031
TITLE: **Vaccination** against ticks (Boophilus spp.): the experience with the Bm86-based **vaccine** GavacTM.
AUTHOR(S): **de la Fuente, J.**; Rodriguez, M.; Montero,

C.; Redondo, M.; Garcia-Garcia, J. C.; Mendez, L.; Serrano, E.; Valdes, M.; Enriquez, A.; Canales, M.; Ramos, E.; Boue, O.; Machado, H.; Lleonart, R.
CORPORATE SOURCE: Division of Mammalian Cell Genetics, Centro de Ingenieria Genetica y Biotecnologia, Havana, Cuba
SOURCE: Genetic Analysis: Biomolecular Engineering (1999), 15(3-5), 143-148
CODEN: GEANF4; ISSN: 1050-3862
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB The control of tick infestations and the transmission of tick-borne diseases remain a challenge for the cattle industry in tropical and subtropical areas of the world. Traditional control methods have been only partially successful and the parasites continue to result in significant losses for the cattle industry. Recently, **vaccines** contg. the recombinant B. microplus gut antigen Bm86 have been developed. Our **vaccine** formulation (GavacTM; Heber Biotec S.A., Havana, Cuba) has been registered and is com. available in Cuba, Colombia, Dominican Republic, Brazil and Mexico. New and previously published results with this **vaccine** are presented. In controlled pen trials, GavacTM has been effective for the control of artificial infestations of B. annulatus, B. decoloratus and chem.-sensitive and resistant B. microplus strains from Australia, Africa, America and Iran. In controlled field trials in Cuba, Brazil, Argentina and Mexico, Gavac has shown a 55-100% efficacy in the control of B. microplus infestations in grazing cattle 12-36 wk after the first **vaccination**. Field trials under prodn. conditions have been conducted in Cuba, Colombia, Brazil and Mexico in pure and cross-bred cattle herds. The application of GavacTM has increased the time between acaricide treatments by an av. of 32 days (P=0.0005) resulting in important savings for the cattle industry. In Cuba, a cost-effectiveness anal. was conducted in more than 260,000 animals. The cost-effectiveness anal. showed a 60% redn. in the no. of acaricide treatments, together with the control of tick infestations and transmission of babesiosis, which resulted in savings of 23.4 animal-1 year-1. These results clearly demonstrate the advantage of **vaccination** and support the application of Gavac for the control of Boophilus spp. infestations.
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 14 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:490019 HCAPLUS
DOCUMENT NUMBER: 132:34282
TITLE: Molecular basis for **vaccine** development against the ehrlichial pathogen Anaplasma marginale
AUTHOR(S): Palmer, G. H.; Rurangirwa, F. R.; **Kocan, K. M.** ; Brown, W. C.
CORPORATE SOURCE: Vector-bome Diseases, Washington State University, Pullman, WA, 99164-7040, USA
SOURCE: Parasitology Today (1999), 15(7), 281-286
CODEN: PATOE2; ISSN: 0169-4758
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal; General Review

LANGUAGE: English
AB A review with refs. Anaplasma marginale is a tick-transmitted ehrlichial pathogen causing severe morbidity and mortality in livestock on six continents. Development of safe effective **vaccines** would be greatly facilitated by identification of the protective **immune** mechanisms and by understanding how the pathogen evades **immune** effectors to establish persistent infection. In this article, the authors review recent progress in identifying how defined epitopes induce protective **immunity** and the role of antigenic variation in these epitopes as a mechanism of persistence.
REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 15 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:316297 HCAPLUS
DOCUMENT NUMBER: 131:125168
TITLE: Mapping of the Antigenic Regions of Streptokinase in Humans after Streptokinase Therapy
AUTHOR(S): Torrens, Isis; Reyes, Osvaldo; Ojalvo, Ariana G.; Seralena, Alina; Chinea, Glay; Cruz, Luis J.; **de la Fuente, Jose**
CORPORATE SOURCE: Division of Pharmaceutical, Centro de Ingenieria Genetica y Biotecnologia, Havana, Cuba
SOURCE: Biochemical and Biophysical Research Communications (1999), 259(1), 162-168
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Streptokinase (SK) is efficaciously used as a thrombolytic drug for the treatment of myocardial infarction. Being a bacterial protein, SK is **immunogenic** in humans. Therefore, resulting from SK therapy, patients become **immunized** and anti-SK antibody (Ab) titers rise post-treatment. High Ab titers might provoke severe **immune** reactions during SK therapy and neutralize SK activity, preventing effective thrombolysis. Spot synthesis combined with peptide library techniques is a useful tool for studying protein-peptide interactions on continuous cellulose membranes. Here, we report on the mapping of antigenic regions of SK using a spot-synthesized peptide library and human total sera from patients receiving SK therapy. All tested samples have high anti-SK Ab titers and most of them show significant SK neutralizing capacity. Individual variations in peptide recognition were detected. However, patients treated with SK tend, in general, to show a common regional binding pattern, including residues 1-20, 130-149, 170-189, and 390-399. This is the first study reporting the probing of a cellulose-bound set of peptides with total human sera. (c) 1999 Academic Press.
REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 16 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:256231 HCAPLUS
DOCUMENT NUMBER: 131:57477
TITLE: Development of enzyme linked **immunosorbent**

assays to measure Bm86 antigen of Boophilus microplus (cattle tick) and to detect anti-Bm86 antibodies in serum samples
AUTHOR(S): Triguero, A.; Blanco, R.; Machado, H.; Rodriguez, M.; **De la Fuente, J.**
CORPORATE SOURCE: Centro de Ingenieria Genetica y Biotecnologia, Sancti Spiritus, Cuba
SOURCE: Biotechnology Techniques (1999), 13(2), 119-125
CODEN: BTECE6; ISSN: 0951-208X
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **immunization** of cattle with the Boophilus microplus Bm86 antigen has been successful for the control of cattle tick infestations. To monitor the Bm86 prodn. process and to measure the anti-Bm86 antibody titers in **vaccinated** cattle, mAb-based ELISA were developed and validated. The development of both **immunol.** methods is essential to obtain a product with high quality and **immunogenic** properties and to monitor the **immunol.** protection induced in **vaccinated** cattle against B. microplus.
REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 17 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:25019 HCAPLUS
DOCUMENT NUMBER: 130:194064
TITLE: Comparison of surface proteins of Anaplasma **marginale** grown in tick cell culture, tick salivary glands, and cattle
AUTHOR(S): Barbet, A. F.; Blentlinger, R.; Yi, Jooyoung; Lundgren, A. M.; **Blouin, E. F.**; **Kocan, K. M.**
CORPORATE SOURCE: Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32611-0880, USA
SOURCE: Infection and Immunity (1999), 67(1), 102-107
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Anaplasma **marginale**, a tick-borne rickettsial pathogen of cattle, infects bovine erythrocytes, resulting in mild to severe hemolytic disease that causes economic losses in domestic livestock worldwide. Recently, the Virginia isolate of A. **marginale** was propagated in a continuous tick cell line, **IDE8**, derived from embryonic Ixodes scapularis. Development of A. **marginale** in cell culture was morphol. similar to that described previously in ticks. In order to evaluate the potential of the cell culture-derived organisms for use in future research or as an antigen for serol. tests and **vaccines**, the extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. **marginale** and the bovine erythrocytic stage, currently the source of A. **marginale** antigen, was detd. Structural conservation on the tick salivary-gland stage was also examd. Monoclonal and monospecific antisera against MSPs 1

through 5, initially characterized against erythrocyte stages, also reacted with A. **marginale** from cell culture and tick salivary glands. MSP1a among geog. A. **marginale** isolates is variable in size because of different nos. of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. **marginale** maintained the same-size MSP1a as that found on the Virginia isolate of A. **marginale** in bovine erythrocytes and tick salivary glands. Although differences were obsd. in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by two-dimensional gel electrophoresis, during continuous passage in culture. These data show that MSPs of erythrocyte-stage A. **marginale** are present on culture stages and may be structurally conserved during continuous culture. The presence of all current candidate diagnostic and **vaccine** antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and **vaccines** against anaplasmosis.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 18 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:772785 HCAPLUS
DOCUMENT NUMBER: 130:178054
TITLE: Expression of fimbriae of enterotoxigenic Escherichia coli K99 in different E. coli K12 strains
AUTHOR(S): Sosa, Angela; Bosulto, Roberto; Ramon, Jose A.; **De la Fuente, Jose**
CORPORATE SOURCE: Div. Desarrolo Biofarmaceutico, Cent. Ing. Genet. Biotecnol., Havana, Cuba
SOURCE: Biotecnologia Aplicada (1998), 15(3), 183-187
CODEN: BTAPEP; ISSN: 0864-4551
PUBLISHER: Sociedad Iberolatinoamericana de Biotecnologia Aplicada a la Salud
DOCUMENT TYPE: Journal
LANGUAGE: Spanish

AB The fimbriae are used for **immunization** of lambs, pigs and calves against gastrointestinal infection with Escherichia coli K99. Due to low expression levels of fimbriae in natural strains, it is attractive to produce these fimbriae by recombinant technol. However, it is difficult to achieve a stable expression in E. coli K12, because of the lysis and the plasmid instability caused by accumulation of the fimbriae inside the bacterium. We previously reported the cloning of gene fanC on the plasmid pUC19. In this work, we report cloning of the complete operon under its own regulatory region on the plasmid pUC19. This genetic construction was evaluated in 14 strains of E. coli K12, and several differences were found in terms of efficiency of transformation, growth, and levels of expression of fimbriae (detd. by a specific ELISA) in Luria-Bertani, min. and saline media. We also discovered that the .DELTA.(ara-leu) deletion favored expression of fimbriae. These observations permit one to select an appropriate host which is an important step in the large-scale prodn. of the fimbriae.

L6 ANSWER 19 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:711553 HCAPLUS
DOCUMENT NUMBER: 130:80085

TITLE: The repertoire of Anaplasma **marginale** antigens recognized by CD4+ T-lymphocyte clones from protectively **immunized** cattle is diverse and includes major surface protein 2 (MSP-2) and MSP-3

AUTHOR(S): Brown, Wendy C.; Zhu, Daming; Shkap, Varda; McGuire, Travis C.; **Blouin, Edmour F.**; **Kocan, Katherine M.**; Palmer, Guy H.

CORPORATE SOURCE: Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, 99164, USA

SOURCE: Infection and Immunity (1998), 66(11), 5414-5422
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Major surface proteins of Anaplasma **marginale** are **vaccine** candidates. We recently demonstrated that **immunization** of calves with outer membranes of the Florida strain of A. **marginale** resulted in protective **immunity** that correlated with a memory CD4+ T-lymphocyte response specific for major surface protein 1 (MSP-1), MSP-2, and MSP-3. As **immunogens**, these proteins have been shown to induce complete or partial protection against homologous challenge. To further define the T helper (Th) cell response to these and other A. **marginale** antigens and to det. conservation of Th cell epitopes among genetically distinct A. **marginale** strains, Th cell clones obtained prior to challenge from three **immunized** calves were characterized for antigen-specific responses. Nine distinct antigenic profiles were defined by 11 Th cell clones derived by stimulation with the Florida strain. Several clones responded to MSP-2, MSP-3, or both. All of these MSP-2-or MSP-3-specific clones and the majority of other clones that did not respond to MSPs recognized all bovine blood-passaged strains of A. **marginale**. These results demonstrate conservation of certain Th cell epitopes between MSP-2 and MSP-3 and show that Th cell epitopes in MSP-2, MSP-3, and undefined antigens are conserved among strains of A. **marginale**. Of seven clones that responded to the blood-passaged Virginia strain, two did not recognize antigen prepd. from this strain cultured in tick cells, suggesting differences in the antigenic compn. between these stages. Anal. of the cytokines expressed by the Th cells revealed that all clones expressed gamma interferon and tumor necrosis factor alpha, and most coexpressed interleukin-4. Our results provide a rationale for identifying Th cell epitopes conserved among different strains of A. **marginale** for inclusion in a nucleic acid or recombinant protein **vaccine**.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 20 OF 32 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1998:469649 HCAPLUS

DOCUMENT NUMBER: 129:243755

TITLE: The development of a semi-automated latex agglutination test for the detection of antibodies to Anaplasma **marginale** using a cell culture-derived antigen

AUTHOR(S): Rodgers, S. J.; Saliki, J. T.; **Blouin, E. F.**

; **Kocan, K. M.**
CORPORATE SOURCE: Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater, OK, 74076-7001, USA
SOURCE: Annals of the New York Academy of Sciences (1998), 849(Tropical Veterinary Medicine), 282-292
CODEN: ANYAA9; ISSN: 0077-8923
PUBLISHER: New York Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Serol. diagnosis of anaplasmosis is currently done by the complement-fixation, ELISA, and card agglutination tests. These tests have utilized A. **marginale** harvested from bovine erythrocytes as antigen which is often contaminated with erythrocyte stroma. We are currently testing A. **marginale** propagated in a Ixodes scapularis cell line as antigen for serol. tests. In this study, we report the use of the cell culture-derived A. **marginale** as antigen for development of a rapid, semi-automated latex agglutination test. Dild. serum and latex (polystyrene microspheres), sensitized with cell culture-derived A. **marginale** proteins, were dispensed into 96-well microtiter plates. An initial reading of light transmission was recorded by a computer-interfaced scanning autoreader. After 30 min, the plates were mixed and read a second time, recording the delta % light transmittance. The sensitized latex microspheres (latex) agglutinated in the presence of A. **marginale** antibodies, thus producing an increase in light transmittance. In preliminary tests, 724/977 of the sera were pos. for A. **marginale** antibodies with an apparent agreement of 83.3% when compared with the complement-fixation test. Sensitization and sera diln. buffers were shown to have a marked effect on the sensitivity and specificity of this assay. Results will be presented on the optimization of buffers and the testing of sera from exptl. and field-infected cattle.

L6 ANSWER 21 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:469648 HCAPLUS
DOCUMENT NUMBER: 129:243754
TITLE: Use of tick cell culture-derived Anaplasma **marginale** antigen in a competitive ELISA for serodiagnosis of anaplasmosis
AUTHOR(S): Saliki, Jeremiah T.; **Blouin, Edmour F.**; Rodgers, Sandy J.; **Kocan, Katherine M.**
CORPORATE SOURCE: Oklahoma Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, 74078, USA
SOURCE: Annals of the New York Academy of Sciences (1998), 849(Tropical Veterinary Medicine), 273-281
CODEN: ANYAA9; ISSN: 0077-8923
PUBLISHER: New York Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Anaplasma **marginale** was propagated in a continuous tick cell line and detergent-solubilized infected cells were used as antigen in a competitive ELISA (C-ELISA) for detection of Anaplasma-specific antibody in bovine sera. Pos. control sera competed well (.gtoreq.35% inhibition)

with an A. **marginale**-specific monoclonal antibody for binding to this antigen, while neg. sera failed to compete (<35% inhibition). The C-ELISA was compared to the std. complement-fixation test (CFT) using 2,208 bovine sera. Overall, C-ELISA was more sensitive than CFT (24.9% vs. 9.4%), mainly because CFT yielded "suspicious" or "anti-complementary" results in 10.5% of the sera and also failed to identify several **vaccinated** and carrier cattle that were C-ELISA-pos. The apparent agreement between CFT and C-ELISA was 89.6% and the kappa value was 0.6. These results show that this C-ELISA would be a suitable replacement of the CFT as the std. test for detection of A. **marginale** antibody.

L6 ANSWER 22 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:440759 HCAPLUS
DOCUMENT NUMBER: 129:201851
TITLE: Adjuvant and **immunostimulating** properties of the recombinant Bm86 protein expressed in Pichia pastoris
AUTHOR(S): Garcia-Garcia, Jose C.; Soto, Alejandro; Nigro, Fabian; Mazza, Marcela; Joglar, Marisdania; Hechevarria, Maidel; Lamberti, Jorge; **De La Fuente, Jose**
CORPORATE SOURCE: Mammalian Cell Genetics Division, Centro de Ingenieria Genetica y Biotecnologia, Havana, Cuba
SOURCE: Vaccine (1998), 16(9/10), 1053-1055
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cattle tick Boophilus microplus has remained a latent problem to the cattle industry. The recombinant **vaccine** GAVAC against the cattle tick has proved its efficacy and, conveniently, combined with the use of chems. could be the soln. to this problem. As this **vaccine** is based in the recombinant concealed antigen Bm86, it has to be given periodically to the animal to maintain an adequate level of antibodies. Some other com. available **vaccines** for cattle also have to be given periodically, which creates the possibility of combining **vaccines** for cattle. In an attempt to evaluate the possible interactions of the Bm86 with other **vaccine** antigens, a potent stimulatory effect was demonstrated of the recombinant Bm86 on the humoral **immune** response to the recombinant Hepatitis B surface antigen in mice, and to the inactivated-Infectious Bovine Rhinotracheitis virus in cattle. These results make the Bm86 antigen expressed in Pichia pastoris a good candidate for combining **vaccines** for cattle because of its dual role, **immunogen** and adjuvant.

L6 ANSWER 23 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:43747 HCAPLUS
DOCUMENT NUMBER: 128:139537
TITLE: Effect of particulation on the **immunogenic** and protective properties of the recombinant Bm86 antigen expressed in Pichia pastoris
AUTHOR(S): Garcia-Garcia, Jose C.; Montero, Carlos; Rodriguez, Manuel; Soto, Alejandro; Redondo, Miguel; Valdes, Mario; Mendez, Luis; **De La Fuente, Jose**

CORPORATE SOURCE: Mammalian Cell Genetics Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba
SOURCE: Vaccine (1998), 16(4), 374-380
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The recombinant Bm86 tick antigen expressed in Pichia pastoris is obtained in a highly particulated form, as a distinguish feature of this expression system. This particulated protein, the active principle of the recombinant **vaccine** Gavac against the cattle tick, have shown high **immunogenic** and protective properties, probably assocd. with its own characteristics. To evaluate the effects of particulation on the properties of Bm86, three groups of calves were **immunized** with particulated or non-particulated recombinant Bm86 and the anti-Bm86 antibody response detd. Animals were challenged with a controlled tick infestation and the protective capacities of both proteins assessed. Humoral **immune** response and protection in cattle **vaccinated** with the particulated antigen were higher. These expts. suggested that particulation of the Bm86 expressed in P. pastoris is an important feature for the protective properties of the antigen in **vaccine** prepns.

L6 ANSWER 24 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:672056 HCAPLUS
DOCUMENT NUMBER: 127:326650
TITLE: Secretion of biologically active recombinant human erythropoietin in mammalian cell culture
AUTHOR(S): Garcia del Barco, Diana; Rodriguez, Alina; Rodriguez, Elsa; Tamayo, Caridad; Lleonart, Ricardo; Aguirre, Alina; **de la Fuente, Jose**
CORPORATE SOURCE: Mammalian Cell Genetics Division, Center Genetic Engineering Biotechnology, Havana, 6, Cuba
SOURCE: Biotecnologia Aplicada (1995), 12(3), 165-166
CODEN: BTAPEP; ISSN: 0864-4551
PUBLISHER: Sociedad Iberolatinoamericana de Biotecnologia Aplicada a la Salud
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant human erythropoietin (hEPO) was detected after transient transfection of CHO cells with an expression plasmid contg. full length cDNA of hEPO cloned from fetal kidneys. A stable transformed line of CHO was established. The rhEPO was partially purified by affinity chromatog. on Blue Sepharose and was detected by either a com. EIA or in **immunodots** with a rabbit heteroserum against a peptide of hEPO. Purifn. of rhEPO yielded a reproducible, more than 90% purity product. Thus, the authors achieved secretion of biol. active rhEPO in CHO cells.

L6 ANSWER 25 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:302176 HCAPLUS
DOCUMENT NUMBER: 126:342483
TITLE: Large-scale production in Pichia pastoris of the recombinant **vaccine** Gavac against cattle tick

AUTHOR(S): Canales, Mario; Enriquez, Antonio; Ramos, Eduardo; Cabrera, Deborah; Dandie, Hubert; Soto, Alejandro; Falcon, Viviana; Rodriguez, Manuel; **De La Fuente, Jose**
CORPORATE SOURCE: Division of Technological Development, Center for Genetic Engineering and Biotechnology, Havana, Cuba
SOURCE: Vaccine (1997), 15(4), 414-422
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A gene coding for the Bm86 tick protein was recently cloned, expressed in Pichia pastoris and shown to induce an **immunol.** response in cattle against ticks. Moreover, the Gavac **vaccine** (Heber Biotec S.A., Havana, Cuba), which contains this recombinant protein, has proved to control the Boophilus microplus populations under field conditions. This paper describes the development and large-scale prodn. of this **vaccine**, the efficacy of the resulting product and the strategy followed in designing its prodn. plant. The prodn. plant fulfills biosafety requirements and GMP.

L6 ANSWER 26 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:276425 HCAPLUS
DOCUMENT NUMBER: 126:248588
TITLE: Method of growing rickettsiae in Ixodes scapularis tick cell culture and preparing antigens and **vaccines** of rickettsiae
INVENTOR(S): Munderloh, Ulrike G.; Kurtti, Timothy J.; **Kocan, Katherine M.; Blouin, Edmour F.**; Ewing, Sidney A.
PATENT ASSIGNEE(S): Regents of the University of Minnesota, USA; Oklahoma State University
SOURCE: PCT Int. Appl., 89 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9708296	A1	19970306	WO 1996-US13594	19960823
W: AL, AM, AT, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5869335	A	19990209	US 1995-519599	19950825
AU 9668559	A1	19970319	AU 1996-68559	19960823
BR 9610681	A	19990703	BR 1996-10681	19960823
PRIORITY APPLN. INFO.:			US 1995-519599	19950825
			WO 1996-US13594	19960823

AB The methods of the invention provide for culture of microorganisms such as Anaplasma **marginale**, Ehrlichia canis, and Rickettsia rickettsii. A method of the invention involves incubating a rickettsia with an I. scapularis tick cell culture in a culture medium under reduced O and increased CO2 at a sufficient temp. until growth of the rickettsia is detected. The culture medium comprises a medium suitable for the growth of invertebrate cells supplemented with an org. buffer. The cell culture method can be used in large-scale prodn. of rickettsia contg. products useful in diagnostic assays and **vaccine** prepns. In one example, A. **marginale**, which causes anaplasmosis in cattle, was grown in I. scapularis cell culture, and then antigens were prepd. for use in **vaccine** prepn. and for diagnostic assays. In other examples, R. rickettsii was grown in **IDE8** tick cell line culture to study the growth of the spotted fever group of rickettsia and E. canis was propagated in **IDE8** tick cell culture.

L6 ANSWER 27 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:85269 HCAPLUS
DOCUMENT NUMBER: 124:172972
TITLE: Production of active anti-CD6 chimeric **immunoglobulins** in the milk of transgenic mice
AUTHOR(S): Limonta, Jose; Pedraza, Alicia; Faxas, Maria E.; Lleonart, Ricardo; Castro, Fidel O.; Garcia, Carlos A.; Gavilondo, Jorge V.; **De la Fuente, Jose**
CORPORATE SOURCE: Division Mammalian Cell Genetics, Center Genetic Engineering and Biotechnology, Havana, 10600, Cuba
SOURCE: Biotecnol. Apl. (1995), 12(2), 84
CODEN: BTAPEP; ISSN: 0864-4551
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Evidence is presented that transgenic female mice can produce active mouse/human chimeric antibodies in milk.

L6 ANSWER 28 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:615847 HCAPLUS
DOCUMENT NUMBER: 123:134294
TITLE: Fate of the heterologous DNA transferred by spermatozoa to murine myeloma-spermatozoa hybrids, and mouse embryos
AUTHOR(S): Aguirre, A.; Duenas, M.; Falcon, V.; Baranovsky, N.; Gavilondo, J.; **De La Fuente, J.**; Castro, F. O.
CORPORATE SOURCE: Mammalian Cell Genetics Division, Centro de Ingenieria Genetica y Biotecnologia, Havana, Cuba
SOURCE: Transgenics (1995), 1(5), 541-52
CODEN: TADTEF
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Spermatozoa from mice can assoc. and internalize exogenous DNA and transfer it to oocytes at fertilization. However, attempts to produce germline transgenic animals using spermatozoa as vectors have repeatedly failed to lead to mosaic transgenic animals or embryos. We investigated the fate of DNA mols. transferred by mouse spermatozoa to murine myeloma cells after polyethyene glycol-mediated fusion, in order to avoid the zona

pellucida, or to oocytes after in vitro fertilization. Roughly 10% of myeloma cells fused to spermatozoa. Biotinylated pCH110 plasmid DNA was detected in the hybrids by **immunoelectron** microscopy. .beta.-Galactosidase was detected by **immunoelectron** microscopy and **immunofluorescence** with monoclonal antibodies, and its biol. activity was confirmed by histochem. X-gal staining. Expression of the gene was transient, and attempts to obtain stably transformed myeloma cells using the plasmid pSV2gpt failed. Oocytes were fertilized with spermatozoa loaded with the plasmid pA327. Plasmid rescue was achieved from total embryonic DNA extd. from 2-cell but not from 4-cell embryos after digestion, ligation, and electroporation in Escherichia coli XL1 blue cells. The frequency of rescue was 1 .times. 10-4 colonies per .mu.g of electroporated DNA. Our results suggested that the DNA carried by spermatozoa was transferred to both myeloma and oocytes, but was lost or degraded during the initial cell cycles and did not contribute to the genome of the targeted cell. These findings could explain why transgenic mosaic embryos and animals, but not germ line transgenics, have been produced using spermatozoa as vectors of the foreign DNA.

L6 ANSWER 29 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:653512 HCAPLUS
DOCUMENT NUMBER: 115:253512
TITLE: Cell-specific expression of the interferon alpha and beta genes
AUTHOR(S): **De la Fuente, J.**
CORPORATE SOURCE: Agrup. Genet. Celulas Mamiferos, Cent. Ing. Genet. Biotecnol., Havana, Cuba
SOURCE: Biotecnol. Apl. (1990), 7(1), 22-31
CODEN: BTAPEP
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Spanish
AB A review with 39 refs. discussing the role of differential genetic transcription in controlling the cellular specificity of interferon expression, and the properties of the 5'-flanking region responsible for the induction of .beta.-interferon.

L6 ANSWER 30 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:551608 HCAPLUS
DOCUMENT NUMBER: 111:151608
TITLE: Synthesis and secretion of the hepatitis B virus surface antigen in mammalian cells
AUTHOR(S): Perez, A.; Rodriguez, R.; Leonard, R.; Guillen, I.; Hernandez, L.; Hernandez, E.; Santizo, C.; **De la Fuente, J.**; Herrera, L.
CORPORATE SOURCE: Cent. Ing. Genet. Biotecnol., Havana, Cuba
SOURCE: Interferon Biotecnol. (1988), 5(3), 223-8
CODEN: INTBEB; ISSN: 0258-9222
DOCUMENT TYPE: Journal
LANGUAGE: Spanish
AB Infection by hepatitis B virus (HBV) is one of the most serious health problems of the human population. The use of mammalian cells in culture to produce an HBV **vaccine** offers a no. of attractive features in comparison to the use of other cell substrates. Recombinant hepatitis B surface antigen (HBsAg) was produced in CHO cells using the DHFR/MTX

amplification system. A prodn. of 1 .mu.g HBsAg/106 cells/day was obtained; the recombinant HBsAg was characterized by electron microscopy and was indistinguishable from plasma-derived HBsAg particles.

L6 ANSWER 31 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:144344 HCAPLUS
DOCUMENT NUMBER: 108:144344
TITLE: Detection of Anaplasma marginale-infected tick vectors by using a cloned DNA probe
AUTHOR(S): Goff, Will; Barbet, Anthony; Stiller, Daivd; Palmer, Guy; Knowles, Donald; **Kocan, Katherine**; Gorham, John; McGuire, Travis
CORPORATE SOURCE: US Dep. Agric., Washington State Univ., Pullman, WA, 99164, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1988), 85(3), 919-23
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Anaplasmosis is the most widely distributed of several important tick-borne diseases that constrain cattle prodn. throughout much of the world. Evaluation of the effectiveness of disease control strategies that integrate **vaccination** with tick control requires the ability to monitor tick and cattle infection rates. To detect A. marginale in ticks and bovine erythrocytes, a 2-kilobase DNA fragment from a cloned A. marginale gene coding for a surface protein having a Mr of 105,000 was prepd. and evaluated as a probe. The probe was species specific and detected A. marginale DNA derived from infected bovine erythrocytes and adult Dermacentor ticks infected either as nymphs or adults. Tick infection was confirmed by microscopy and test feeding on a susceptible calf. The sensitivity of the probe is suitable for detecting infected ticks in exptl. and field epizootiol. studies.

L6 ANSWER 32 OF 32 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1986:32786 HCAPLUS
DOCUMENT NUMBER: 104:32786
TITLE: Presence of common antigens, including major surface protein epitopes, between the cattle (intraerythrocytic) and tick stages of Anaplasma marginale
AUTHOR(S): Palmer, Guy H.; **Kocan, Katherine M.**; Barron, Selwyn J.; Hair, Jakie A.; Barbet, Anthony F.; Davis, William C.; McGuire, Travis C.
CORPORATE SOURCE: Dep. Vet. Microbiol. Pathol., Washington State Univ., Pullman, WA, 99164-7040, USA
SOURCE: Infect. Immun. (1985), 50(3), 881-6
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Epitopes of major surface proteins of the intraerythrocytic cattle stage of A. marginale were demonstrated in the midgut stage of the organism within the infective tick host Dermacentor andersoni. These proteins were common to all A. marginale isolates tested and at all stages of parasitemia. Sera from cattle **immunized** with the tick midgut stage of A. marginale **immunopptd**. multiple-erythrocyte-stage

proteins, as demonstrated by SDS-polyacrylamide gel electrophoresis. The major proteins recognized (primarily >14 and <200 kilodaltons [kDa]) included 2 major-erythrocyte-stage surface proteins of 36 and 105 kDa. To confirm the presence of common tick and erythrocyte A. marginale antigens with the **immunized** cattle sera, the 36-kDa erythrocyte-stage protein was purified by monoclonal **immunoaffinity** chromatog. and an ELISA was developed, based on the purified protein. All sera from cattle **immunized** with tick-stage A. marginale and cattle infected with various isolates of A. marginale developed antibodies to the 36-kDa protein. The potential **immunoprophylactic,** diagnostic, and epidemiol. value of the major epitopes common to both the invertebrate and mammalian stages of A. marginale, esp. the 36-kDa protein, is discussed.

```
show files
File 155:MEDLINE(R) 1966-2002/Aug W1
File   5:Biosis Previews(R) 1969-2002/Aug W1
         (c) 2002 BIOSIS
File  10:AGRICOLA 70-2002/Aug
         (c) format only 2002 The Dialog Corporation
File  34:SciSearch(R) Cited Ref Sci 1990-2002/Aug W2
         (c) 2002 Inst for Sci Info
File  50:CAB Abstracts 1972-2002/Jul
         (c) 2002 CAB International
File  71:ELSEVIER BIOBASE 1994-2002/Aug W1
         (c) 2002  Elsevier Science B.V.
File  73:EMBASE 1974-2002/Aug W1
         (c) 2002 Elsevier Science B.V.
File  76:Life Sciences Collection 1982-2002/Jul
         (c) 2002 Cambridge Sci Abs
File 144:Pascal 1973-2002/Aug W1
         (c) 2002 INIST/CNRS
File 162:CAB HEALTH 1983-2002/Jul
         (c) 2002 CAB INTERNATIONAL
File 185:Zoological Record Online(R) 1978-2002/Aug
         (c) 2002 BIOSIS
File 440:Current Contents Search(R) 1990-2002/Aug 09
         (c) 2002 Inst for Sci Info
?ds

Set     Items   Description
S1        100   (MSP1A OR MSP1(W)A) AND (VACCIN? OR IMMUN?)
S2         24   RD (unique items)
?t2/3 ab/1-24

 2/AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13349698   22103551   PMID: 12107477
  Detection of the Anaplasma centralevaccine strain and specific
differentiation from Anaplasma marginale in vaccinated and infected
cattle.
  Shkap V; Molad T; Fish L; Palmer G H
  Division of Parasitology, Kimron Veterinary Institute. P.O. Box 12, Bet
Dagan 50250, Israel, shkap@agri.huji.ac.il
  Parasitology research (Germany) Jun 2002, 88 (6) p546-52, ISSN
0932-0113  Journal Code: 8703571
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: In Process
  Bovine anaplasmosis caused by the intraerythrocytic rickettsia Anaplasma
marginale is the most prevalent tick-borne disease of cattle worldwide. The
most efficient method to control anaplasmosis is by vaccination using
live Anaplasma centrale, a closely related species or subspecies of low
pathogenicity that is capable of inducing significant protection against
the more virulent A. marginale. In the present study, we applied PCR assays
to detect and discriminate field infection with A. marginale from A.
centrale persistently infected vaccinates . Direct and one-stage nested
PCR were based on A. centrale mbp58 specific sequence, with the assay
sensitivity level of 0.00001% for nested PCR performed in a single
amplification step. Size polymorphism in the A. marginale msp1 alpha gene
among strains was used to design a PCR capable of discriminating between
the Israel T and NT strains of A. marginale and the encoded MSP1a size
polymorphism was confirmed by immunoprecipitation. The detection of A.
```

centrale in 72% of vaccinated field-grazing cattle clearly indicated that the majority of vaccinated cattle remain carriers. A. marginalewas detected in 64% of these vaccinated cattle, demonstrating that, as expected, natural transmission occurs within the endemic region. The lack of severe A. marginaleoutbreaks in this region, despite ongoing transmission, is consistent with protection being provided by widespread vaccination with A. centrale.

2/AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13128182 21588118 PMID: 11730800
Major surface protein 1a effects tick infection and transmission of Anaplasma marginale.
de la Fuente J; Garcia-Garcia J C; Blouin E F; McEwen B R; Clawson D; Kocan K M
Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, USA. jose delafuente@yahoo.com
International journal for parasitology (England) Dec 2001, 31 (14) p1705-14, ISSN 0020-7519 Journal Code: 0314024
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Anaplasma marginale, an ehrlichial pathogen of cattle and wild ruminants, is transmitted biologically by ticks. A developmental cycle of A. marginale occurs in a tick that begins in gut cells followed by infection of salivary glands, which are the site of transmission to cattle. Geographic isolates of A. marginale vary in their ability to be transmitted by ticks. In these experiments we studied transmission of two recent field isolates of A. marginale, an Oklahoma isolate from Wetumka, OK, and a Florida isolate from Okeechobee, FL, by two populations of Dermacentor variabilis males obtained from the same regions. The Florida and Oklahoma tick populations transmitted the Oklahoma isolate, while both tick populations failed to transmit the Florida isolate. Gut and salivary gland infections of A. marginale, as determined by quantitative PCR and microscopy, were detected in ticks exposed to the Oklahoma isolate, while these tissues were not infected in ticks exposed to the Florida isolate. An adhesion-recovery assay was used to study adhesion of the A. marginale major surface protein (MSP) 1a to gut cells from both tick populations and cultured tick cells. We demonstrated that recombinant Escherichia coli expressing Oklahoma MSP1a adhered to cultured and native D. variabilis gut cells, while recombinant E. coli expressing the Florida MSP1a were not adherent to either tick cell population. The MSP1a of the Florida isolate of A. marginale, therefore, was unable to mediate attachment to tick gut cells, thus inhibiting salivary gland infection and transmission to cattle. This is the first report of MSP1a being responsible for effecting infection and transmission of A. marginale by Dermacentor spp. ticks. The mechanism of tick infection and transmission of A. marginale is important in formulating control strategies and development of improved vaccines for anaplasmosis.

2/AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13046762 21954225 PMID: 11955782
A msp1alpha polymerase chain reaction assay for specific detection and differentiation of Anaplasma marginale isolates.

Lew A E; Bock R E; Minchin C M; Masaka S
Queensland Department of Primary Industries, Agency for Food and Fibre Sciences, c/o Animal Research Institute, Locked Mail Bag No. 4, Qld 4105, Moorooka, Australia
Veterinary microbiology (Netherlands) May 24 2002, 86 (4) p325-35, ISSN 0378-1135 Journal Code: 7705469
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process
Anaplasma marginale is the causative agent of bovine anaplasmosis, a disease which can be protected by vaccination with the less pathogenic Anaplasma species, A. centrale. Currently, there is no polymerase chain reaction (PCR) assay available which differentiates between different species of Anaplasma or which can differentiate isolates of A. marginale within outbreaks and between different countries. A molecular test specific for A. marginale would be ideal for the identification of Anaplasma species in wild ruminants, as possible reservoirs of anaplasmosis, and to differentiate between A. marginale from A. centrale. A PCR assay was designed to amplify the major surface protein 1alpha gene of the rickettsial bovine pathogen, A. marginale both as an inter- and intra-specific test. The test did not amplify A. centrale or A. ovis, and discriminated A. marginale by amplifying repeat regions within the msp1alpha gene which vary in number between many isolates. The nested A. marginale amplicons varied in size from 630 to 1190bp representing one to eight internal repeats. All 22 Australian isolates tested amplified a 630bp product (one repeat) in contrast to all 19 non-Australian isolates tested. Eight sequences from Australian isolates from different geographical regions confirmed the conserved nature of the Australian A. marginale msp1alpha genes. The Australian 'repeat unit' MSP1a deduced amino acid sequence has been designated as Australian type 1. The msp1alpha PCR method developed here enabled the amplification and comparison of A. marginale isolates originating from North and South America, Africa, Israel and Australia. The method is sensitive and specific for A. marginale. Although additional msp1alpha products were amplified from at least two Australian isolates, the results suggest limited introduction of A. marginale into Australia.

2/AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12983729 21673992 PMID: 11814681
Conservation of major surface protein 1 genes of Anaplasma marginale during cyclic transmission between ticks and cattle.
Bowie Michael V; de la Fuente Jose; Kocan Katherine M; Blouin Edmour F; Barbet Anthony F
Department of Pathobiology, University of Florida, PO Box 110880, Gainesville, FL 32611-0880, USA. mbowie@ufl.edu
Gene (Netherlands) Jan 9 2002, 282 (1-2) p95-102, ISSN 0378-1119 Journal Code: 7706761
Contract/Grant No.: AI45580-01S1; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Bovine anaplasmosis is a rickettsial disease of world-wide economic importance caused by Anaplasma marginale. Several major surface proteins with conserved gene sequences have been examined as potential candidates for vaccines and/or diagnostic assays. Major surface protein 1 (MSP1) is composed of polypeptides MSP1a and MSP1b. MSP1a is expressed from the

single copy gene msp1 alpha and MSP1b is expressed by members of the msp1 beta multigene family. In order to determine if the msp1 genes are conserved, primers specific for msp1 alpha, msp1 beta(1), and msp1 beta(2) genes were synthesized and used to amplify msp1 sequences of A. marginale from tick cell cultures, from cattle during acute and chronic infections and from salivary glands of Dermacentor variabilis. Protein sequences of MSP1a , MSP1b(1) and MSP1b(2) were conserved during the life cycle of the parasite. No amino acid changes were observed in MSP1a . However, small variations were observed in the MSP1b(1) and MSP1b(2) protein sequences, which could be attributed to recombination, selection for sub-populations of A. marginale in the vertebrate host and/or PCR errors. Several isolate-specific sequences were also observed. Based on the information obtained in this study, the MSP1 protein appears to be fairly well conserved and a potential vaccine candidate.

2/AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12932353 21820001 PMID: 11831437
Evolution and function of tandem repeats in the major surface protein 1a of the ehrlichial pathogen Anaplasma marginale.
de La Fuente J; Garcia-Garcia J C; Blouin E F; Rodriguez S D; Garcia M A; Kocan K M
Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater 74078, USA. jose delafuente@yahoo.com
Anim Health Res Rev (England) Dec 2001, 2 (2) p163-73, ISSN 1466-2523 Journal Code: 101083072
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The major surface protein (MSP) 1a of the ehrlichial cattle pathogen Anaplasma marginale, encoded by the single-copy gene msp1alpha, has been shown to have a neutralization-sensitive epitope and to be an adhesin for bovine erythrocytes and tick cells. msp1alpha has been found to be a stable genetic marker for the identification of geographic isolates of A. marginale throughout development in acutely and persistently infected cattle and in ticks. The molecular weight of MSP1a varies among geographic isolates of A. marginale because of a varying number of tandemly repeated peptides of 28-29 amino acids. Variation in the sequence of the tandem repeats occurs within and among isolates, and may have resulted from evolutionary pressures exerted by ligand-receptor and host-parasite interactions. These repeated sequences include markers for tick transmissibility that may be important in the identification of ehrlichial pathogens because they may influence control strategies and the design of subunit vaccines .

2/AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12576072 21481962 PMID: 11598059
CD4(+) T lymphocytes from calves immunized with Anaplasma marginale major surface protein 1 (MSP1), a heteromeric complex of MSP1a and MSP1b, preferentially recognize the MSP1a carboxyl terminus that is conserved among strains.
Brown W C; Palmer G H; Lewin H A; McGuire T C
Program in Vector-Borne Diseases, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164, USA. wbrown@vetmed.wsu.edu

Infection and immunity (United States) Nov 2001, 69 (11) p6853-62, ISSN 0019-9567 Journal Code: 0246127
Contract/Grant No.: R01-AI44005; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Native major surface protein 1 (MSP1) of the ehrlichial pathogen Anaplasma marginale induces protective immunity in calves challenged with homologous and heterologous strains. MSP1 is a heteromeric complex of a single MSP1a protein covalently associated with MSP1b polypeptides, of which at least two (designated MSP1F1 and MSP1F3) in the Florida strain are expressed. Immunization with recombinant MSP1a and MSP1b alone or in combination fails to provide protection. The protective immunity in calves immunized with native MSP1 is associated with the development of opsonizing and neutralizing antibodies, but CD4(+) T-lymphocyte responses have not been evaluated. CD4(+) T lymphocytes participate in protective immunity to ehrlichial pathogens through production of gamma interferon (IFN-gamma), which promotes switching to high-affinity immunoglobulin G (IgG) and activation of phagocytic cells to produce nitric oxide. Thus, an effective vaccine for A. marginale and related organisms should contain both T- and B-lymphocyte epitopes that induce a strong memory response that can be recalled upon challenge with homologous and heterologous strains. This study was designed to determine the relative contributions of MSP1a and MSP1b proteins, which contain both variant and conserved amino acid sequences, in stimulating memory CD4(+) T-lymphocyte responses in calves immunized with native MSP1. Peripheral blood mononuclear cells and CD4(+) T-cell lines from MSP1- immunized calves proliferated vigorously in response to the immunizing strain (Florida) and heterologous strains of A. marginale. The conserved MSP1-specific response was preferentially directed to the carboxyl-terminal region of MSP1a , which stimulated high levels of IFN-gamma production by CD4(+) T cells. In contrast, there was either weak or no recognition of MSP1b proteins. Paradoxically, all calves developed high titers of IgG antibodies to both MSP1a and MSP1b polypeptides. These findings suggest that in calves immunized with MSP1 heteromeric complex, MSP1a -specific T lymphocytes may provide help to MSP1b-specific B lymphocytes. The data provide a basis for determining whether selected MSP1a CD4(+) T-lymphocyte epitopes and selected MSP1a and MSP1b B-lymphocyte epitopes presented on the same molecule can stimulate a protective immune response.

2/AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11125885 21135802 PMID: 11239934
Differential adhesion of major surface proteins 1a and 1b of the ehrlichial cattle pathogen Anaplasma marginale to bovine erythrocytes and tick cells.

de la Fuente J; Garcia-Garcia J C; Blouin E F; Kocan K M
Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, USA. jose delafuente@yahoo.com

International journal for parasitology (England) Feb 2001, 31 (2) p145-53, ISSN 0020-7519 Journal Code: 0314024
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Anaplasma marginale is a tick-borne ehrlichial pathogen of cattle for which six major surface proteins (MSPs) have been described. The MSP1

complex, a heterodimer composed of MSP1a and MSP1b, was shown to induce a protective immune response in cattle and both proteins have been identified as putative adhesins for bovine erythrocytes. In this study the role of MSP1a and MSP1b as adhesins for bovine erythrocytes and tick cells was defined. msp1alpha and msp1beta1 genes from the Oklahoma isolate of A. marginale were cloned and expressed in Escherichia coli K-12 under the control of endogenous and tac promoters for both low and high level protein expression. Expression of the recombinant polypeptides was confirmed and localised on the surface of transformed E. coli. The adhesion properties of MSP1a and MSP1b were determined by allowing recombinant E. coli expressing these surface polypetides to react with bovine erythrocytes, Dermacentor variabilis gut cells and cultured tick cells derived from embryonic Ixodes scapularis. Adhesion of the recombinant E. coli to the three cell types was determined using recovery adhesion and microtiter haemagglutination assays, and by light and electron microscopy. MSP1a was shown by all methods tested to be an adhesin for bovine erythrocytes and both native and cultured tick cells. In contrast, recombinant E. coli expressing MSP1b adhered only to bovine erythrocytes and not to tick cells. When low expression vectors were used, single E. coli expressing MSP1a was seen adhered to individual tick cells while reaction of tick cells with the E. coli/ MSP1a /high expression vector resulted in adhesion of multiple bacteria per cell. With electron microscopy, fusion of E. coli cell membranes expressing MSP1a or MSP1b with erythrocyte membranes was observed, as well as fusion of tick cell membranes with E. coli membranes expressing MSP1a . These studies demonstrated differential adhesion for MSP1a and MSP1b for which MSP1a is an A. marginale adhesin for both bovine erythrocytes and tick cells while MSP1b is an adhesin only for bovine erythrocytes. The role of the MSP1 complex, therefore, appears to vary among vertebrate and invertebrate hosts.

2/AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10641494 20187463 PMID: 10722587
Expression of polymorphic msp1beta genes during acute anaplasma Marginale rickettsemia.
Camacho-Nuez M; de Lourdes Munoz M; Suarez C E; McGuire T C; Brown W C; Palmer G H
Departamento de Genetica y Biologia Molecular, CINVESTAV-IPN, D.F. 07000, Mexico.
Infection and immunity (UNITED STATES) Apr 2000, 68 (4) p1946-52, ISSN 0019-9567 Journal Code: 0246127
Contract/Grant No.: RO1 AI44005; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Immunization of cattle with native MSP1 induces protection against Anaplasma marginale. The native immunogen is composed of a single MSP1a protein and multiple, undefined MSP1b polypeptides. In addition to the originally sequenced gene, designated msp1beta(F1), we identified three complete msp1beta genes in the Florida strain: msp1beta(F2), msp1beta(F3), and msp1beta(F4). Each of these polymorphic genes encodes a structurally unique MSP1b protein, and unique transcripts can be identified during acute A. marginale rickettsemia. The structural polymorphism is clustered in discrete variable regions, and each MSP1b protein results from a unique mosaic of five variable regions. Although each of the MSP1b proteins in the Florida strain contains epitopes recognized by serum antibody induced by protective immunization with the native MSP1 complex, the variable

regions also include epitopes expressed by some but not all of the MSP1b proteins. These data support testing recombinant vaccines composed of the multiple antigenically and structurally unique MSP1b proteins combined with MSP1a in order to mimic the efficacy of native MSP1 immunization .

2/AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10313159 99307208 PMID: 10377129
Biased immunoglobulin G1 isotype responses induced in cattle with DNA expressing msp1a of Anaplasma marginale.
Arulkanthan A; Brown W C; McGuire T C; Knowles D P
Program in Vector-Borne Diseases, Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, Washington 99164, USA.
Infection and immunity (UNITED STATES) Jul 1999, 67 (7) p3481-7,
ISSN 0019-9567 Journal Code: 0246127
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Immunization with the native major surface protein 1 (MSP1) (a heterodimer containing disulfide and noncovalently bonded polypeptides designated MSP1a and MSP1b) of the erythrocytic stage of Anaplasma marginale conferred protection against homologous challenge (G. H. Palmer, A. F. Barbet, W. C. Davis, and T. C. McGuire, Science 231:1299-1302, 1986). The MSP1a polypeptide possesses a conserved neutralization-sensitive epitope. In the present study, the immune response to DNA-mediated immunization using msp1a was studied. The plasmid pVCL/ MSP1a , which encodes the complete msp1a gene of A. marginale under the control of human cytomegalovirus immediate-early enhancer/promoter and intron A, was constructed. The immune responses elicited by immunization with pVCL/ MSP1a into cardiotoxin-induced regenerating muscle were evaluated in mice and cattle. Antibody reactive with native MSP1a was detected in pooled sera of immunized BALB/c mice 3 weeks following primary immunization . Two calves seronegative for A. marginale were immunized four times, at weeks 0, 3, 7, and 13, with pVCL/ MSP1a . By 8 weeks, both calves responded to MSP1a with an antibody titer of 1:100, which peaked at 1:1,600 and 1:800 by 16 weeks after the initial immunization . Interestingly, immunoblotting with anti-immunoglobulin G1 (anti-IgG1) and anti-IgG2 specific monoclonal antibodies revealed a restricted IgG1 anti- MSP1a response in both animals. T-lymphocyte lines, established after the fourth immunization , proliferated specifically against A. marginale homogenate and purified MSP1 in a dose-dependent manner. These data provide a basis for an immunization strategy to direct bovine immune responses by using DNA vaccine vectors containing single or multiple genes encoding major surface proteins of A. marginale.

2/AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10101047 99081729 PMID: 9864202
Comparison of surface proteins of Anaplasma marginale grown in tick cell culture, tick salivary glands, and cattle.
Barbet A F; Blentlinger R; Yi J; Lundgren A M; Blouin E F; Kocan K M
Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville USA. abarbet@nervm.nerdc.ufl.edu
Infection and immunity (UNITED STATES) Jan 1999, 67 (1) p102-7,
ISSN 0019-9567 Journal Code: 0246127

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Anaplasma marginale, a tick-borne rickettsial pathogen of cattle, infects bovine erythrocytes, resulting in mild to severe hemolytic disease that causes economic losses in domestic livestock worldwide. Recently, the Virginia isolate of A. marginale was propagated in a continuous tick cell line, IDE8, derived from embryonic Ixodes scapularis. Development of A. marginale in cell culture was morphologically similar to that described previously in ticks. In order to evaluate the potential of the cell culture-derived organisms for use in future research or as an antigen for serologic tests and vaccines , the extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. marginale and the bovine erythrocytic stage, currently the source of A. marginale antigen, was determined. Structural conservation on the tick salivary-gland stage was also examined. Monoclonal and monospecific antisera against MSPs 1 through 5, initially characterized against erythrocyte stages, also reacted with A. marginale from cell culture and tick salivary glands. MSP1a among geographic A. marginale isolates is variable in size because of different numbers of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. marginale maintained the same-size MSP1a as that found on the Virginia isolate of A. marginale in bovine erythrocytes and tick salivary glands. Although differences were observed in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by two-dimensional gel electrophoresis, during continuous passage in culture. These data show that MSPs of erythrocyte-stage A. marginale are present on culture stages and may be structurally conserved during continuous culture. The presence of all current candidate diagnostic and vaccine antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and vaccines against anaplasmosis.

2/AB/11 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10928519 BIOSIS NO.: 199799549664
MSP1-reactive T cells in individuals in malaria endemic Solomon area and in non- immune Japanese.
AUTHOR: Fu Jun; Kunimatsu Mitoshi; Leafasia Judson L; Kere Nathan; Tanabe Kazuyuki; Hirayama Kenji; Ishii Akira; Saitoh-Ito Atsuko; Susaki Makoto; Ohta Nobuo(a)
AUTHOR ADDRESS: (a)Dep. Med. Zool., Nagoya City Univ. Med. Sch., Kawasumi, Mizuhocho, Mizuhoku, Nagoya 467**Japan
JOURNAL: Parasitology International 46 (1):p7-16 1997
ISSN: 1383-5769
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We analyzed functions and specificities of human helper T cells reactive to the N-terminal blocks of MSP1 , a major merozoite surface glycoprotein of Plasmodium falciparum. Since human T cells showed proliferative response to MSP1 in vitro regardless of their previous infection with malaria, we established blastoid T cell lines reactive to the N-terminal 6 blocks (M1/6) of MSP1 from both the Solomon's population (malaria-exposed) and the Japanese (non-exposed) donors. T cell lines from non-exposed donors preferentially recognized the 6th block from the N-terminus, and those T cells recognized only a few epitope peptides

expressed in the block. On the other hand, the putative immune T cells of the Solomon's donors recognized both the 3rd and 6th blocks, and a large number of peptides in the 6th block induced positive responses of the immune T cells. Specificities of the responding T cells were, thus, not identical between the two donor groups. It seemed unlikely that such difference was caused by some particular constitution of HLA-class II alleles in the two ethnically different populations, because we observed similar results even when comparisons were made under the same HLA-DR allorestriction. Significantly elevated expression of CD30 in the non-exposed T cells suggested that T cells from those two groups were functionally different. Together with those results, infection with falciparum malaria induces human T cell response to MSP1, of which specificity and function seem to be substantially different from those in malaria non-exposed donors.

1997

2/AB/12 (Item 1 from file: 10)
DIALOG(R)File 10:AGRICOLA

3452986 20465519 Holding Library: AGL
Putative adhesins of Anaplasma marginale: major surface polypeptides 1a and 1b
McGarey, D.J. Barbet, A.F.; Palmer, G.H.; McGuire, T.C.; Allred, D.R.
Jacksonville State University, Jacksonville, AL.
Washington, D.C., American Society for Microbiology
Infection and immunity. Oct 1994. v. 62 (10) p. 4594-4601.
ISSN: 0019-9567
DNAL CALL NO: QR1.I57
Language: English
Genes for the MSP1a and MSP1b subunits of the Anaplasma marginale surface antigen complex MSP1 were previously cloned and expressed in Escherichia coli. We report here the localization of MSP1a and MSP1b polypeptides on the surface of recombinant E. coli by using a live cell indirect immunofluorescent antibody assay. Recombinant E. coli cells expressing the msp1alpha gene or the msp1beta gene encoding the MSP1a and MSP1b polypeptide subunits, respectively, were shown by a culture recovery adhesion assay and by direct microscopic examination to specifically adhere to bovine erythrocytes. This adhesion was more than additive when both genes were coexpressed in a single recombinant construct. Similarly, these recombinants hemagglutinated bovine erythrocytes in a microtiter hemagglutination assay. Inhibition of recombinant E. coli adhesion to bovine erythrocytes and hemagglutination inhibition were observed in the presence of homologous monospecific polyclonal antiserum raised against purified MSP1a or MSP1b polypeptide. These data suggest that the MSP1a and MSP1b polypeptides have functions as adhesins on A. marginale initial bodies, probably during erythrocyte invasion.

2/AB/13 (Item 2 from file: 10)
DIALOG(R)File 10:AGRICOLA

3159654 92018019 Holding Library: AGL
Detection of Anaplasma marginale rickettsemia prior to onset of clinical signs by using an antigen capture enzyme-linked immunosorbent assay
Trueblood, E.S. McGuire, T.C.; Palmer, G.H.
Washington State University, Pullman, WA
Washington, D.C. : American Society for Microbiology.

Journal of clinical microbiology. July 1991. v. 29 (7) p. 1542-1544.
ISSN: 0095-1137 CODEN: JCMIDW
DNAL CALL NO: QR46.J6
Language: English

An antigen capture enzyme-linked immunosorbent assay was developed by using monoclonal antibodies to conserved epitopes on the Anaplasma marginale MSP1a surface protein. The assay sensitivity was 1.1 (+/-0.5)% parasitized erythrocytes, and all infected cattle were detected prior to development of 2.0%-parasitized erythrocytes. Positive tests preceded the onset of anemia by a mean of 2 days. The assay was specific for anaplasmosis, as demonstrated by nonreactivity with other common hemoparasitic pathogens.

2/AB/14 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09637695 Genuine Article#: 429UQ Number of References: 40
Title: Molecular phylogeny and biogeography of North American isolates of Anaplasma marginale (Rickettsiaceae : Ehrlichieae) (ABSTRACT AVAILABLE)
Author(s): de la Fuente J (REPRINT) ; Van den Bussche RA; Kocan KM
Corporate Source: Oklahoma State Univ,Coll Vet Med, Dept Vet Pathobiol,Stillwater//OK/74078 (REPRINT); Oklahoma State Univ,Coll Vet Med, Dept Vet Pathobiol,Stillwater//OK/74078; Oklahoma State Univ,Dept Zool & Collect Vertebrates,Stillwater//OK/74708
Journal: VETERINARY PARASITOLOGY, 2001, V97, N1 (MAY 9), P65-76
ISSN: 0304-4017 Publication date: 20010509
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE
Abstract: Anaplasma marginale (A. marginale) is a tick-borne ehrlichial pathogen of cattle that causes the disease anaplasmosis. Six major surface proteins (MSPs) have been identified on A. marginale from cattle and ticks of which three, MSP1a , MSP4 and MSP5, are from single genes and do not vary within isolates. The other three, MSP1b, MSP2 and MSP3, are from multigene families and may vary antigenically in persistently infected cattle. Several geographic isolates have been identified in the United States which differ in morphology, protein sequence and antigenic properties. An identifying characteristic of A. marginale isolates is the molecular weight of MSP1a which varies in size among isolates due to different numbers of tandemly repeated 28-29 amino acid peptides. For these studies, genes coding for A. marginale MSP1a and MSP4, msp1 alpha and msp4, respectively, from nine North American isolates were sequenced for phylogenetic analysis. The phylogenetic analysis strongly supports the existence of a south-eastern clade of A. marginale comprised of Virginia and Florida isolates. Analysis of 16S rDNA fragment sequences from the A. marginale tick vector, Dermacentor variabilis, from various areas of the United States was used to evaluate possible vector-parasite co-evolution. Our phylogenetic analysis supports identity between the most parsimonious tree from the A. marginale MSP gene data and the tree that reflected the western and eastern clades of D. variabilis. These phylogenetic analyses provide information that may be important to consider when developing control strategies for anaplasmosis in the United States.

2/AB/15 (Item 1 from file: 50)
DIALOG(R)File 50:CAB Abstracts

04169253 CAB Accession Number: 20023007511
Major surface protein 1a effects tick infection and transmission of Anaplasma marginale.
Fuente, J. de la; Garcia-Garcia, J. C.; Blouin, E. F.; McEwen, B. R.; Clawson, D.; Kocan, K. M.
Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, USA.
International Journal for Parasitology vol. 31 (14): p.1705-1714
Publication Year: 2001
ISSN: 0020-7519 --
Language: English
Document Type: Journal article
Anaplasma marginale, an ehrlichial pathogen of cattle and wild ruminants, is transmitted biologically by ticks. A developmental cycle of A. marginale occurs in a tick that begins in gut cells followed by infection of salivary glands, which are the site of transmission to cattle. Geographic isolates of A. marginale vary in their ability to be transmitted by ticks. In these experiments we studied transmission of 2 recent field isolates of A. marginale, an Oklahoma isolate from Wetumka, OK, and a Florida isolate from Okeechobee, FL, by 2 populations of Dermacentor variabilis males obtained from the same regions. The Florida and Oklahoma tick populations transmitted the Oklahoma isolate, while both tick populations failed to transmit the Florida isolate. Gut and salivary gland infections of A. marginale, as determined by quantitative PCR and microscopy, were detected in ticks exposed to the Oklahoma isolate, while these tissues were not infected in ticks exposed to the Florida isolate. An adhesion-recovery assay was used to study adhesion of the A. marginale major surface protein (MSP) 1a to gut cells from both tick populations and cultured tick cells. We demonstrated that recombinant Escherichia coli expressing Oklahoma MSP1a adhered to cultured and native D. variabilis gut cells, while recombinant E. coli expressing the Florida MSP1a were not adherent to either tick cell population. The MSP1a of the Florida isolate of A. marginale, therefore, was unable to mediate attachment to tick gut cells, thus inhibiting salivary gland infection and transmission to cattle. This is the first report of MSP1a being responsible for effecting infection and transmission of A. marginale by Dermacentor spp. ticks. The mechanism of tick infection and transmission of A. marginale is important in formulating control strategies and development of improved vaccines for anaplasmosis. 31 ref.

2/AB/16 (Item 2 from file: 50)
DIALOG(R)File 50:CAB Abstracts

04032386 CAB Accession Number: 20013037538
Differential adhesion of major surface proteins 1a and 1b of the ehrlichial cattle pathogen Anaplasma marginale to bovine erythrocytes and tick cells.
Fuente, J. de la; Garcia-Garcia, J. C.; Blouin, E. F.; Kocan, K. M.
Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, USA.
International Journal for Parasitology vol. 31 (2): p.145-153
Publication Year: 2001
ISSN: 0020-7519 --
Language: English
Document Type: Journal article
Anaplasma marginale is a tick-borne ehrlichial pathogen of cattle for which six major surface proteins (MSPs) have been described. The MSP1 complex, a heterodimer composed of MSP1a and MSP1b, was shown to induce

a protective immune response in cattle and both proteins have been identified as putative adhesins for bovine erythrocytes. In this study the role of MSP1a and MSP1b as adhesins for bovine erythrocytes and tick cells was defined. msp1 alpha and msp1 beta 1 genes from the Oklahoma isolate of A. marginale were cloned and expressed in Escherichia coli K-12 under the control of endogenous and tac promoters for both low and high level protein expression. Expression of the recombinant polypeptides was confirmed and localized on the surface of transformed E. coli. The adhesion properties of MSP1a and MSP1b were determined by allowing recombinant E. coli expressing these surface polypeptides to react with bovine erythrocytes, Dermacentor variabilis gut cells and cultured tick cells derived from embryonic Ixodes scapularis. Adhesion of the recombinant E. coli to the three cell types was determined using recovery adhesion and microtitre haemagglutination assays, and by light and electron microscopy. MSP1a was shown by all methods tested to be an adhesin for bovine erythrocytes and both native and cultured tick cells. In contrast, recombinant E. coli expressing MSP1b adhered only to bovine erythrocytes and not to tick cells. When low expression vectors were used, single E. coli expressing MSP1a was seen adhering to individual tick cells while reaction of tick cells with the E. coli/ MSP1a /high expression vector resulted in adhesion of multiple bacteria per cell. With electron microscopy, fusion of E. coli cell membranes expressing MSP1a or MSP1b with erythrocyte membranes was observed, as well as fusion of tick cell membranes with E. coli membranes expressing MSP1a . These studies demonstrated differential adhesion for MSP1a and MSP1b for which MSP1a is an A. marginale adhesin for both bovine erythrocytes and tick cells while MSP1b is an adhesin only for bovine erythrocytes. The role of the MSP1 complex, therefore, appears to vary among vertebrate and invertebrate hosts. 28 ref.

2/AB/17 (Item 3 from file: 50)
DIALOG(R)File 50:CAB Abstracts

03918963 CAB Accession Number: 20000507033
Biased immunoglobulin G1 isotype responses induced in cattle with DNA expressing msp1a of Anaplasma marginale.
Appudurai Arulkanthan; Brown, W. C.; McGuire, T. C.; Knowles, D. P.
Program in Vector-Borne Diseases, Department of Veterinary Microbiology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164, USA.
Infection and Immunity vol. 67 (7): p.3481-3487
Publication Year: 1999
ISSN: 0019-9567 --
Language: English
Document Type: Journal article
Immunization with the native major surface protein 1 (MSP1) (a heterodimer containing disulfide and noncovalently bonded polypeptides designated MSP1a and MSP1b) of the erythrocytic stage of Anaplasma marginale conferred protection against homologous challenge (Palmer, G. H., et al., Science (1986) 231, 1299-1309). The MSP1a polypeptide possesses a conserved neutralization-sensitive epitope. In the present study, the immune response to DNA-mediated immunization using msp1a was studied. The plasmid pVCL/ MSP1a , which encodes the complete msp1a gene of A. marginale under the control of human cytomegalovirus immediate-early enhancer/promoter and intron A, was constructed. The immune responses elicited by immunization with pVCL/ MSP1a into cardiotoxin-induced regenerating muscle were evaluated in mice and cattle. Antibody reactive with native MSP1a was detected in pooled sera of immunized BALB/c mice 3 weeks following primary immunization . Two

calves seronegative for A. marginale were immunized four times, at weeks 0, 3, 7, and 13, with pVCL/ MSP1a . By 8 weeks, both calves responded to MSP1a with an antibody titre of 1:100, which peaked at 1:1,600 and 1:800 by 16 weeks after the initial immunization . Interestingly, immunoblotting with anti- immunoglobulin G1 (anti-IgG1) and anti-IgG2 specific monoclonal antibodies revealed a restricted IgG1 anti- MSP1a response in both animals. T-lymphocyte lines, established after the fourth immunization , proliferated specifically against A. marginale homogenate and purified MSP1 in a dose-dependent manner. These data provide a basis for an immunization strategy to direct bovine immune responses by using DNA vaccine vectors containing single or multiple genes encoding major surface proteins of A. marginale. 60 ref.

2/AB/18 (Item 4 from file: 50)
DIALOG(R)File 50:CAB Abstracts

03375549 CAB Accession Number: 970802388
Statistical analysis of highly skewed immune response data.
McGuinness, D.; Bennett, S.; Riley, E.
Institute of Cell, Animal and Population Biology, Division of Biological Sciences, University of Edinburgh, Kings Buildings, Edinburgh EH9 3JT, UK.
Journal of Immunological Methods vol. 201 (1): p.99-114
Publication Year: 1997
ISSN: 0022-1759 --
Language: English
Document Type: Journal article
Methods of statistical analysis for highly skewed immune response data are considered. Using resampling techniques, applied to several actual datasets of ELISA assay data, the robustness of normal parametric methods (including t tests and linear regression) is assessed. Despite the skewness of the transformed data, it was demonstrated that these methods are quite robust, depending on the number of observations, type of analysis and severity of skewness. Bootstrap resampling was used to provide a valid alternative method of analysis, both for checking normal parametric analysis and as a direct method of analysis. This combined approach was illustrated by analysing real data to test for association between human serum antibodies to Plasmodium falciparum merozoite surface proteins (MSP1 and MSP2) and resistance to clinical malaria, and to confirm the protective effect of antibodies to MSP1 . A similar protective effect was demonstrated for some antibodies to MSP2. 25 ref.

2/AB/19 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

01881738 2001242782
CD4SUP+ T lymphocytes from calves immunized with Anaplasma marginale major surface protein 1 (MSP1), a heteromeric complex of MSP1a and MSP1b, preferentially recognize the MSP1a carboxyl terminus that is conserved among strains
Brown W.C.; Palmer G.H.; Lewin H.A.; McGuire T.C.
ADDRESS: W.C. Brown, Dept. of Veterinary Microbiology, Washington State University, Pullman, WA 99164-7040, United States
EMAIL: wbrown@vetmed.wsu.edu
Journal: Infection and Immunity, 69/11 (6853-6862), 2001, United States
CODEN: INFIB
ISSN: 0019-9567
DOCUMENT TYPE: Article

LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 49

Native major surface protein 1 (MSP1) of the ehrlichial pathogen Anaplasma marginale induces protective immunity in calves challenged with homologous and heterologous strains. MSP1 is a heteromeric complex of a single MSP1a protein covalently associated with MSP1b polypeptides, of which at least two (designated MSP1F1 and MSP1F3) in the Florida strain are expressed. Immunization with recombinant MSP1a and MSP1b alone or in combination fails to provide protection. The protective immunity in calves immunized with native MSP1 is associated with the development of opsonizing and neutralizing antibodies, but CD4SUP+ T-lymphocyte responses have not been evaluated. CD4SUP+ T lymphocytes participate in protective immunity to ehrlichial pathogens through production of gamma interferon (IFN-gamma), which promotes switching to high-affinity immunoglobulin G (IgG) and activation of phagocytic cells to produce nitric oxide. Thus, an effective vaccine for A. marginale and related organisms should contain both T- and B-lymphocyte epitopes that induce a strong memory response that can be recalled upon challenge with homologous and heterologous strains. This study was designed to determine the relative contributions of MSP1a and MSP1b proteins, which contain both variant and conserved amino acid sequences, in stimulating memory CD4SUP+ T-lymphocyte responses in calves immunized with native MSP1. Peripheral blood mononuclear cells and CD4SUP+ T-cell lines from MSP1- immunized calves proliferated vigorously in response to the immunizing strain (Florida) and heterologous strains of A. marginale. The conserved MSP1-specific response was preferentially directed to the carboxyl-terminal region of MSP1a , which stimulated high levels of IFN-gamma production by CD4SUP+ T cells. In contrast, there was either weak or no recognition of MSP1b proteins. Paradoxically, all calves developed high titers of IgG antibodies to both MSP1a and MSP1b polypeptides. These findings suggest that in calves immunized with MSP1 heteromeric complex, MSP1a -specific T lymphocytes may provide help to MSP1b-specific B lymphocytes. The data provide a basis for determining whether selected MSP1a CD4SUP+ T-lymphocyte epitopes and selected MSP1a and MSP1b B-lymphocyte epitopes presented on the same molecule can stimulate a protective immune response.

2/AB/20 (Item 1 from file: 76)
DIALOG(R)File 76:Life Sciences Collection

02671251 5288496
CD4 super(+) T Lymphocytes from Calves Immunized with Anaplasma marginale Major Surface Protein 1 (MSP1), a Heteromeric Complex of MSP1a and MSP1b, Preferentially Recognize the MSP1a Carboxyl Terminus That Is Conserved among Strains
Brown, W.C.; Palmer, G.H.; Lewin, H.A.; McGuire, T.C.
Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040.
Infection and Immunity vol. 69, no. 11, pp. 6853-6862 (2001)
ISSN: 0019-9567
DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH
SUBFILE: Microbiology Abstracts B: Bacteriology; Microbiology Abstracts A: Industrial & Applied Microbiology; Immunology Abstracts

Native major surface protein 1 (MSP1) of the ehrlichial pathogen Anaplasma marginale induces protective immunity in calves challenged with homologous and heterologous strains. MSP1 is a heteromeric complex of a single MSP1a protein covalently associated with MSP1b polypeptides, of which at least two (designated MSP1F1 and MSP1F3) in the Florida strain are

expressed. Immunization with recombinant MSP1a and MSP1b alone or in combination fails to provide protection. The protective immunity in calves immunized with native MSP1 is associated with the development of opsonizing and neutralizing antibodies, but CD4 super(+) T-lymphocyte responses have not been evaluated. CD4 super(+) T lymphocytes participate in protective immunity to ehrlichial pathogens through production of gamma interferon (IFN- gamma), which promotes switching to high-affinity immunoglobulin G (IgG) and activation of phagocytic cells to produce nitric oxide. Thus, an effective vaccine for A. marginale and related organisms should contain both T- and B-lymphocyte epitopes that induce a strong memory response that can be recalled upon challenge with homologous and heterologous strains. This study was designed to determine the relative contributions of MSP1a and MSP1b proteins, which contain both variant and conserved amino acid sequences, in stimulating memory CD4 super(+) T-lymphocyte responses in calves immunized with native MSP1. Peripheral blood mononuclear cells and CD4 super(+) T-cell lines from MSP1- immunized calves proliferated vigorously in response to the immunizing strain (Florida) and heterologous strains of A. marginale. The conserved MSP1-specific response was preferentially directed to the carboxyl-terminal region of MSP1a , which stimulated high levels of IFN- gamma production by CD4 super(+) T cells. In contrast, there was either weak or no recognition of MSP1b proteins. Paradoxically, all calves developed high titers of IgG antibodies to both MSP1a and MSP1b polypeptides. These findings suggest that in calves immunized with MSP1 heteromeric complex, MSP1a -specific T lymphocytes may provide help to MSP1b-specific B lymphocytes. The data provide a basis for determining whether selected MSP1a CD4 super(+) T-lymphocyte epitopes and selected MSP1a and MSP1b B-lymphocyte epitopes presented on the same molecule can stimulate a protective immune response.

2/AB/21 (Item 2 from file: 76)
DIALOG(R)File 76:Life Sciences Collection

02471709 4704090
Expression of polymorphic msp1 beta genes during acute Anaplasma marginale rickettsemia
Camacho Nuez, M.; De Lourdes Munoz, M.; Suarez, C.E.; McGuire, T.C.; Brown, W.C.; Palmer, G.H.
Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040, USA
Infection and Immunity vol. 68, no. 4, pp. 1946-1952 (2000)
ISSN: 0019-9567
DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH
SUBFILE: Microbiology Abstracts B: Bacteriology

Immunization of cattle with native MSP1 induces protection against Anaplasma marginale. The native immunogen is composed of a single MSP1a protein and multiple, undefined MSP1b polypeptides. In addition to the originally sequenced gene, designated msp1 beta (F1), we identified three complete msp1 beta genes in the Florida strain: msp1 beta (F2), msp1 beta (F3), and msp1 beta (F4). Each of these polymorphic genes encodes a structurally unique MSP1b protein, and unique transcripts can be identified during acute A. marginale rickettsemia. The structural polymorphism is clustered in discrete variable regions, and each MSP1b protein results from a unique mosaic of five variable regions. Although each of the MSP1b proteins in the Florida strain contains epitopes recognized by serum antibody induced by protective immunization with the native MSP1 complex, the variable regions also include epitopes expressed by some but not all of the MSP1b proteins. These data support testing recombinant vaccines

composed of the multiple antigenically and structurally unique MSP1b proteins combined with MSP1a in order to mimic the efficacy of native MSP1 immunization .

2/AB/22 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

15618306 PASCAL No.: 02-0322548
CD4 SUP + T lymphocytes from calves immunized with Anaplasma marginale major surface protein 1 (MSP1), a heteromeric complex of MSP1a and MSP1b, preferentially recognize the mspla carboxyl terminus that is conserved among strains
BROWN Wendy C; PALMER Guy H; LEWIN Harris A; MCGUIRE Travis C
Program in Vector-Borne Diseases, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164, United States; Department of Animal Sciences, University of Illinois, Urbana-Champaign, Illinois 61801, United States
Journal: Infection and immunity, 2001, 69 (11) 6853-6862
Language: English
Native major surface protein 1 (MSP1) of the ehrlichial pathogen Anaplasma marginale induces protective immunity in calves challenged with homologous and heterologous strains. MSP1 is a heteromeric complex of a single MSP1a protein covalently associated with MSP1b polypeptides, of which at least two (designated MSP1F1 and MSP1F3) in the Florida strain are expressed. Immunization with recombinant MSP1a and MSP1b alone or in combination fails to provide protection. The protective immunity in calves immunized with native MSP1 is associated with the development of opsonizing and neutralizing antibodies, but CD4 SUP + T-lymphocyte responses have not been evaluated. CD4 SUP + T lymphocytes participate in protective immunity to ehrlichial pathogens through production of gamma interferon (IFN- gamma), which promotes switching to high-affinity immunoglobulin G (IgG) and activation of phagocytic cells to produce nitric oxide. Thus, an effective vaccine for A. marginale and related organisms should contain both T- and B-lymphocyte epitopes that induce a strong memory response that can be recalled upon challenge with homologous and heterologous strains. This study was designed to determine the relative contributions of MSP1a and MSP1b proteins, which contain both variant and conserved amino acid sequences, in stimulating memory CD4 SUP + T-lymphocyte responses in calves immunized with native MSPI. Peripheral blood mononuclear cells and CD4 SUP + T-cell lines from MSP1- immunized calves proliferated vigorously in response to the immunizing strain (Florida) and heterologous strains of A. marginale. The conserved MSP1-specific response was preferentially directed to the carboxyl-terminal region of MSP1a , which stimulated high levels of IFN-y production by CD4 SUP + T cells. In contrast, there was either weak or no recognition of MSP1b proteins. Paradoxically, all calves developed high titers of IgG antibodies to both MSP1a and MSP1b polypeptides. These findings suggest that in calves immunized with MSP1 heteromeric complex, MSP1a -specific T lymphocytes may provide help to MSP1b-specific B lymphocytes. The data provide a basis for determining whether selected MSP1a CD4 SUP + T-lymphocyte epitopes and selected MSP1a and MSP1b B-lymphocyte epitopes presented on the same molecule can stimulate a protective immune response.

2/AB/23 (Item 1 from file: 185)
DIALOG(R)File 185:Zoological Record Online(R)

02060469 BIOSIS No. 13805000624
Conservation of major surface protein 1 genes of Anaplasma marginale during cyclic transmission between ticks and cattle.
AUTHORS: Bowie Michael V (a); de la Fuente Jose; Kocan Katherine M; Blouin Edmour F; Barbet Anthony F
AUTHORS ADDRESS: (a) Department of Pathobiology, University of Florida, PO Box 110880, Gainesville, FL, 32611-0880, USA
JOURNAL: Gene (Amsterdam) 282(1-2), 9 January 2002: 95-102.
DOCUMENT TYPE: Article; Print
ISSN: 0378-1119
LANGUAGES: English SUMMARY LANGUAGES: English

ABSTRACT: Bovine anaplasmosis is a rickettsial disease of world-wide economic importance caused by Anaplasma marginale. Several major surface proteins with conserved gene sequences have been examined as potential candidates for vaccines and/or diagnostic assays. Major surface protein 1 (MSP1) is composed of polypeptides MSP1a and MSP1b. MSP1a is expressed from the single copy gene msp1 [alpha] and MSP1b is expressed by members of the msp1 [beta] multigene family. In order to determine if the msp1 genes are conserved, primers specific for msp1 [alpha], msp1 [beta]1, and msp1 [beta]2 genes were synthesized and used to amplify msp1 sequences of A. marginale from tick cell cultures, from cattle during acute and chronic infections and from salivary glands of Dermacentor variabilis. Protein sequences of MSP1a , MSP1b1 and MSP1b2 were conserved during the life cycle of the parasite. No amino acid changes were observed in MSP1a . However, small variations were observed in the MSP1b1 and MSP1b2 protein sequences, which could be attributed to recombination, selection for sub-populations of A. marginale in the vertebrate host and/or PCR errors. Several isolate-specific sequences were also observed. Based on the information obtained in this study, the MSP1 protein appears to be fairly well conserved and a potential vaccine candidate.

2/AB/24 (Item 2 from file: 185)
DIALOG(R)File 185:Zoological Record Online(R)

01979888 BIOSIS No. 13700013861
Differential adhesion of major surface proteins 1a and 1b of the ehrlichial cattle pathogen Anaplasma marginale to bovine erythrocytes and tick cells.
AUTHORS: de la Fuente J (a); Garcia Garcia J C; Blouin E F; Kocan K M
AUTHORS ADDRESS: (a) Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, 74078, USA
JOURNAL: International Journal for Parasitology 31(2), February 2001: 145-153.
DOCUMENT TYPE: Article; Print
ISSN: 0020-7519
LANGUAGES: English SUMMARY LANGUAGES: English

ABSTRACT: Anaplasma marginale is a tick-borne ehrlichial pathogen of cattle for which six major surface proteins (MSPs) have been described. The MSP1 complex, a heterodimer composed of MSP1a and MSP1b, was shown to induce a protective immune response in cattle and both proteins have been identified as putative adhesins for bovine erythrocytes. In this study the role of MSP1a and MSP1b as adhesins for bovine erythrocytes and tick cells was defined. msp1[alpha] and msp1[beta]1 genes from the Oklahoma isolate of A. marginale were cloned and expressed in Escherichia coli K-12 under the control of endogenous and tac promoters for both low

and high level protein expression. Expression of the recombinant polypeptides was confirmed and localised on the surface of transformed E. coli. The adhesion properties of MSP1a and MSP1b were determined by allowing recombinant E. coli expressing these surface polypetides to react with bovine erythrocytes, Dermacentor variabilis gut cells and cultured tick cells derived from embryonic Ixodes scapularis. Adhesion of the recombinant E. coli to the three cell types was determined using recovery adhesion and microtiter haemagglutination assays, and by light and electron microscopy. MSP1a was shown by all methods tested to be an adhesin for bovine erythrocytes and both native and cultured tick cells. In contrast, recombinant E. coli expressing MSP1b adhered only to bovine erythrocytes and not to tick cells. When low expression vectors were used, single E. coli expressing MSP1a was seen adhered to individual tick cells while reaction of tick cells with the E. coli/ MSP1a /high expression vector resulted in adhesion of multiple bacteria per cell. With electron microscopy, fusion of E. coli cell membranes expressing MSP1a or MSP1b with erythrocyte membranes was observed, as well as fusion of tick cell membranes with E. coli membranes expressing MSP1a . These studies demonstrated differential adhesion for MSP1a and MSP1b for which MSP1a is an A. marginale adhesin for both bovine erythrocytes and tick cells while MSP1b is an adhesin only for bovine erythrocytes. The role of the MSP1 complex, therefore, appears to vary among vertebrate and invertebrate hosts.

?ds

Set	Items	Description
S1	100	(MSP1A OR MSP1(W)A) AND (VACCIN? OR IMMUN?)
S2	24	RD (unique items)
S3	20	(IDE8 OR IDE(W)8) AND (VACCIN? OR IMMUN?)
S4	19	S3 NOT S2
S5	9	S4 AND (ANAPLASMA? OR MARGINALE?)
S6	9	S5 NOT S2

?t6/3 ab/1-9

6/AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12526184 BIOSIS NO.: 200000279686
Tick cell culture: New approaches for Anaplasma research.
AUTHOR: Kocan K M; Blouin E F; Barbet A F; Saliki J T; McEwen B R; Meeus P F M
AUTHOR ADDRESS: (a)College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, 74078**USA
JOURNAL: In Vitro Cellular & Developmental Biology Animal 36 (3 Part 2):p 5A March, 2000
MEDIUM: print.
CONFERENCE/MEETING: Meeting of the Society for In Vitro Biology World Congress on In Vitro Biology. San Diego, California, USA June 10-15, 2000
ISSN: 1071-2690
RECORD TYPE: Citation
LANGUAGE: English
SUMMARY LANGUAGE: English
2000

6/AB/2 (Item 1 from file: 10)
DIALOG(R)File 10:AGRICOLA

3762537 21993277 Holding Library: AGL

Comparison of surface proteins of Anaplasma marginale grown in tick cell culture, tick salivary glands, and cattle

Barbet, A.F. Blentlinger, R.; Yi, J.; Lundgren, A.M.; Blouin, E.F.; Kocan, K.M.
University of Florida, Gainesville, FL.

Washington, D.C., American Society for Microbiology
Infection and immunity. Jan 1999. v. 67 (1) p. 102-107.
ISSN: 0019-9567
DNAL CALL NO: QR1.I57
Language: English

Anaplasma marginale , a tick-borne rickettsial pathogen of cattle, infects bovine erythrocytes, resulting in mild to severe hemolytic disease that causes economic losses in domestic livestock worldwide. Recently, the Virginia isolate of A. marginale was propagated in a continuous tick cell line, IDE8 , derived from embryonic Ixodes scapularis. Development of A. marginale in cell culture was morphologically similar to that described previously in ticks. In order to evaluate the potential of the cell culture-derived organisms for use in future research or as an antigen for serologic tests and vaccines , the extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. marginale and the bovine erythrocytic stage, currently the source of A. marginale antigen, was determined. Structural conservation on the tick salivary-gland stage was also examined. Monoclonal and monospecific antisera against MSPs 1 through 5, initially characterized against erythrocyte stages, also reacted with A. marginale from cell culture and tick salivary glands. MSP1a among geographic A. marginale isolates is variable in size because of different numbers of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. marginale maintained the same-size MSP1a as that found on the Virginia isolate of A. marginale in bovine erythrocytes and tick salivary glands. Although differences were observed in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by two-dimensional gel electrophoresis, during continuous passage in culture. These data show that MSPs of erythrocyte-stage A. marginale are present on culture stages and may be structurally conserved during continuous culture. The presence of all current candidate diagnostic and vaccine antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and vaccines against anaplasmosis.

6/AB/3 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

07336345 Genuine Article#: 152EV Number of References: 34
Title: Comparison of surface proteins of Anaplasma marginale grown in tick cell culture, tick salivary glands, and cattle (ABSTRACT AVAILABLE)
Author(s): Barbet AF (REPRINT) ; Blentlinger R; Yi JY; Lundgren AM; Blouin EF; Kocan KM
Corporate Source: UNIV FLORIDA,DEPT PATHOBIOL, COLL VET MED, POB 110880/GAINESVILLE//FL/32611 (REPRINT); OKLAHOMA STATE UNIV,COLL VET MED, DEPT ANAT PATHOL & PHARMACOL/STILLWATER//OK/74078
Journal: INFECTION AND IMMUNITY, 1999, V67, N1 (JAN), P102-107
ISSN: 0019-9567 Publication date: 19990100
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
Language: English Document Type: ARTICLE
Abstract: Anaplasma marginale , a tick-borne rickettsial pathogen of cattle, infects bovine erythrocytes, resulting in mild to severe

hemolytic disease that causes economic losses in domestic livestock worldwide. Recently, the Virginia isolate of A. marginale was propagated in a continuous tick cell Line, IDE8 , derived from embryonic Ixodes scapularis. Development of A. marginale in cell culture was morphologically similar to that described previously in ticks. In order to evaluate the potential of the cell culture-derived organisms for use in future research or as an antigen for serologic tests and vaccines , the extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. marginale and the bovine erythrocytic stage, currently the source of A. marginale antigen, was determined. Structural conservation on the tick salivary-gland stage was also examined. Monoclonal and monospecific antisera against MSPs 1 through 5, initially characterized against erythrocyte stages, also reacted with A, marginale from cell culture and tick salivary glands, MSP1a among geographic A. marginale isolates is variable in size because of different numbers of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. marginale maintained the same-size MSP1a as that found on the Virginia Isolate of A. marginale in bovine erythrocytes and tick salivary glands. Although differences were observed in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by two-dimensional gel electrophoresis, during continuous passage in culture, These data show that MSPs of erythrocyte-stage A. marginale are present on culture stages and may be structurally conserved during continuous culture, The presence of all current candidate diagnostic and vaccine antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and vaccines against anaplasmosis.

6/AB/4 (Item 1 from file: 50)
DIALOG(R)File 50:CAB Abstracts

03720519 CAB Accession Number: 990502802
Comparison of surface proteins of Anaplasma marginale grown in tick cell culture, tick salivary glands, and cattle.
Barbet, A. F.; Blentlinger, R.; Yi JooYoung; Lundgren, A. M.; Blouin, E. F.; Kocan, K. M.
Department of Pathobiology, PO Box 110880, University of Florida, Gainesville, FL 32611-0880, USA.
Infection and Immunity vol. 67 (1): p.102-107
Publication Year: 1999
ISSN: 0019-9567 --
Language: English
Document Type: Journal article
Recently, the Virginia isolate of A. marginale was propagated in a continuous tick cell line, IDE8 , derived from embryonic Ixodes scapularis. Development of A. marginale in cell culture was morphologically similar to that described previously in ticks. In order to evaluate the potential of the cell culture-derived organisms for use in future research or as an antigen for serologic tests and vaccines , the extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. marginale and the bovine erythrocytic stage, currently the source of A. marginale antigen, was determined. Structural conservation on the tick salivary-gland stage was also examined. Monoclonal and monospecific antisera against MSPs 1-5, initially characterized against erythrocyte stages, also reacted with A. marginale from cell culture and tick salivary glands. MSP1a among geographic A. marginale isolates is variable in size because of

different numbers of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. marginale maintained the same-size MSP1a as that found on the Virginia isolate of A. marginale in bovine erythrocytes and tick salivary glands. Although differences were observed in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by 2-dimensional gel electrophoresis, during continuous passage in culture. These data show that MSPs of erythrocyte-stage A. marginale are present on culture stages and may be structurally conserved during continuous culture. The presence of all current candidate diagnostic and vaccine antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and vaccines against anaplasmosis. 34 ref.

6/AB/5 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

01044172 1999011702
Comparison of surface proteins of Anaplasma marginale grown in tick cell culture, tick salivary glands, and cattle
Barbet A.F.; Blentlinger R.; Yi J.; Lundgren A.M.; Blouin E.F.; Kocan K.M.
ADDRESS: A.F. Barbet, Department of Pathobiology, University of Florida, P.O. Box 110880, Gainesville, FL 32611-0880, United States
EMAIL: abarbet@nervm.nerdc.ufl.edu
Journal: Infection and Immunity, 67/1 (102-107), 1999, United States
CODEN: INFIB
ISSN: 0019-9567
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 34

Anaplasma marginale , a tick-borne rickettsial pathogen of cattle, infects bovine erythrocytes, resulting in mild to severe hemolytic disease that causes economic losses in domestic livestock worldwide. Recently, the Virginia isolate of A. marginale was propagated in a continuous tick cell line, IDE8 , derived from embryonic Ixodes scapularis. Development of A. marginale in cell culture was morphologically similar to that described previously in ticks. In order to evaluate the potential of the cell culture- derived organisms for use in future research or as an antigen for serologic tests and vaccines , the extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. marginale and the bovine erythrocytic stage, currently the source of A. marginale antigen, was determined. Structural conservation on the tick salivary-gland stage was also examined. Monoclonal and monospecific antisera against MSPs 1 through 5, initially characterized against erythrocyte stages, also reacted with A. marginale from cell culture and tick salivary glands. MSP1a among geographic A. marginale isolates is variable in size because of different numbers of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. marginale maintained the same-size MSP1a as that found on the Virginia isolate of A. marginale in bovine erythrocytes and tick salivary glands. Although differences were observed in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by two- dimensional gel electrophoresis, during continuous passage in culture. These data show that MSPs of erythrocyte-stage A. marginale are present on culture stages and may be structurally conserved during continuous culture. The presence of all current candidate diagnostic and vaccine antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and vaccines against

anaplasmosis.

6/AB/6 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

07609993 EMBASE No: 1999016672
Comparison of surface proteins of Anaplasma marginale grown in tick cell culture, tick salivary glands, and cattle
Barbet A.F.; Blentlinger R.; Yi J.; Lundgren A.M.; Blouin E.F.; Kocan K.M.
A.F. Barbet, Department of Pathobiology, University of Florida, P.O. Box 110880, Gainesville, FL 32611-0880 United States
AUTHOR EMAIL: abarbet@nervm.nerdc.ufl.edu
Infection and Immunity (INFECT. IMMUN.) (United States) 1999, 67/1 (102-107)
CODEN: INFIB ISSN: 0019-9567
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 34

Anaplasma marginale , a tick-borne rickettsial pathogen of cattle, infects bovine erythrocytes, resulting in mild to severe hemolytic disease that causes economic losses in domestic livestock worldwide. Recently, the Virginia isolate of A. marginale was propagated in a continuous tick cell line, IDE8 , derived from embryonic Ixodes scapularis. Development of A. marginale in cell culture was morphologically similar to that described previously in ticks. In order to evaluate the potential of the cell culture- derived organisms for use in future research or as an antigen for serologic tests and vaccines , the extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. marginale and the bovine erythrocytic stage, currently the source of A. marginale antigen, was determined. Structural conservation on the tick salivary-gland stage was also examined. Monoclonal and monospecific antisera against MSPs 1 through 5, initially characterized against erythrocyte stages, also reacted with A. marginale from cell culture and tick salivary glands. MSP1a among geographic A. marginale isolates is variable in size because of different numbers of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. marginale maintained the same-size MSP1a as that found on the Virginia isolate of A. marginale in bovine erythrocytes and tick salivary glands. Although differences were observed in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by two- dimensional gel electrophoresis, during continuous passage in culture. These data show that MSPs of erythrocyte-stage A. marginale are present on culture stages and may be structurally conserved during continuous culture. The presence of all current candidate diagnostic and vaccine antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and vaccines against anaplasmosis.

6/AB/7 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

13962983 PASCAL No.: 99-0145018
Comparison of surface proteins of Anaplasma marginale grown in tick cell culture, tick salivary glands, and cattle
BARBET A F; BLENTLINGER R; YI J; LUNDGREN A M; BLOUIN E F; KOCAN K M

Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, United States; Department of Anatomy, Pathology and Pharmacology, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma, United States
Journal: Infection and immunity, 1999, 67 (1) 102-107
Language: English
Anaplasma marginale , a tick-borne rickettsial pathogen of cattle, infects bovine erythrocytes, resulting in mild to severe hemolytic disease that causes economic losses in domestic livestock worldwide. Recently, the Virginia isolate of A. marginale was propagated in a continuous tick cell line, IDE8 , derived from embryonic Ixodes scapularis, Development of A. marginale in cell culture was morphologically similar to that described previously in ticks. In order to evaluate the potential of the cell culture-derived organisms for use in future research or as an antigen for serologic tests and vaccines , the extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. marginale and the bovine erythrocytic stage, currently the source of A. marginale antigen, was determined. Structural conservation on the tick salivary-gland stage was also examined. Monoclonal and monospecific antisera against MSPs I through 5, initially characterized against erythrocyte stages, also reacted with A. marginale from cell culture and tick salivary glands. MSP1a among geographic A. marginale isolates is variable in size because of different numbers of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. marginale maintained the same-size MSP1a as that found on the Virginia isolate of A. marginale in bovine erythrocytes and tick salivary glands. Although differences were observed in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by two-dimensional gel electrophoresis, during continuous passage in culture. These data show that MSPs of erythrocyte-stage A. marginale are present on culture stages and may be structurally conserved during continuous culture. The presence of all current candidate diagnostic and vaccine antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and vaccines against anaplasmosis.

6/AB/8 (Item 1 from file: 162)
DIALOG(R)File 162:CAB HEALTH

00671040 CAB Accession Number: 990502802
Comparison of surface proteins of Anaplasma marginale grown in tick cell culture, tick salivary glands, and cattle.
Barbet, A. F.; Blentlinger, R.; Yi JooYoung; Lundgren, A. M.; Blouin, E. F.; Kocan, K. M.
Department of Pathobiology, PO Box 110880, University of Florida, Gainesville, FL 32611-0880, USA.
Infection and Immunity vol. 67 (1): p.102-107
Publication Year: 1999
ISSN: 0019-9567
Language: English
Document Type: Journal article
Recently, the Virginia isolate of A. marginale was propagated in a continuous tick cell line, IDE8 , derived from embryonic Ixodes scapularis. Development of A. marginale in cell culture was morphologically similar to that described previously in ticks. In order to evaluate the potential of the cell culture-derived organisms for use in future research or as an antigen for serologic tests and vaccines , the

extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. marginale and the bovine erythrocytic stage, currently the source of A. marginale antigen, was determined. Structural conservation on the tick salivary-gland stage was also examined. Monoclonal and monospecific antisera against MSPs 1-5, initially characterized against erythrocyte stages, also reacted with A. marginale from cell culture and tick salivary glands. MSP1a among geographic A. marginale isolates is variable in size because of different numbers of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. marginale maintained the same-size MSP1a as that found on the Virginia isolate of A. marginale in bovine erythrocytes and tick salivary glands. Although differences were observed in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by 2-dimensional gel electrophoresis, during continuous passage in culture. These data show that MSPs of erythrocyte-stage A. marginale are present on culture stages and may be structurally conserved during continuous culture. The presence of all current candidate diagnostic and vaccine antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and vaccines against anaplasmosis. 34 ref.

6/AB/9 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10135618 References: 34
TITLE: Comparison of surface proteins of Anaplasma marginale grown in tick cell culture, tick salivary glands, and cattle
AUTHOR(S): Barbet AF (REPRINT); Blentlinger R; Yi JY; Lundgren AM; Blouin EF; Kocan KM
AUTHOR(S) E-MAIL: abarbet@nervm.nerdc.ufl.edu
CORPORATE SOURCE: Univ Florida, Coll Vet Med, POB 110880/Gainesville//FL/32611 (REPRINT); Univ Florida, Coll Vet Med, /Gainesville//FL/32611; Oklahoma State Univ, Dept Anat Pathol & Pharmacol, /Stillwater//OK/74078
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1999, V67, N1 (JAN), P102-107
GENUINE ARTICLE#: 152EV
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Anaplasma marginale , a tick-borne rickettsial pathogen of cattle, infects bovine erythrocytes, resulting in mild to severe hemolytic disease that causes economic losses in domestic livestock worldwide. Recently, the Virginia isolate of A. marginale was propagated in a continuous tick cell Line, IDE8 , derived from embryonic Ixodes scapularis. Development of A. marginale in cell culture was morphologically similar to that described previously in ticks. In order to evaluate the potential of the cell culture-derived organisms for use in future research or as an antigen for serologic tests and vaccines , the extent of structural conservation of the major surface proteins (MSPs) between the cell culture-derived A. marginale and the bovine erythrocytic stage, currently the source of A. marginale antigen, was determined. Structural conservation on the tick salivary-gland stage was also examined. Monoclonal and monospecific antisera against MSPs 1 through 5, initially characterized against erythrocyte stages, also reacted with A, marginale from cell culture and tick salivary glands, MSP1a among geographic A.

marginale isolates is variable in size because of different numbers of a tandemly repeated 28- or 29-amino-acid peptide. The cell culture-derived A. marginale maintained the same-size MSP1a as that found on the Virginia Isolate of A. marginale in bovine erythrocytes and tick salivary glands. Although differences were observed in the polymorphic MSP2 antigen between culture and salivary-gland stages, MSP2 did not appear to vary, by two-dimensional gel electrophoresis, during continuous passage in culture, These data show that MSPs of erythrocyte-stage A. marginale are present on culture stages and may be structurally conserved during continuous culture, The presence of all current candidate diagnostic and vaccine antigens suggests that in vitro cultures are a valuable source of rickettsiae for basic research and for the development of improved diagnostic reagents and vaccines against anaplasmosis.
?
?